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FILE 'HOME' ENTERED AT 09:37:23 ON 05 JUL 2005

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(FILE 'HOME' ENTERED AT 11:26:55 ON 05 JUL 2005)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, ...' ENTERED AT 11:27:29 ON 05 JUL 2005

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         5  FILE RDISCLOSURE
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         1  FILE VETB
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      3170  FILE WPIDS
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      3170  FILE WPINDEX
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  FILE 'EMBASE, SCISEARCH, BIOSIS, MEDLINE, CAPLUS, TOXCENTER, BIOTECHNO,
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L3       35760 S (GENE# OR SEQUENCE# OR CLONE# OR POLYNUCLEOTIDE# OR RECOMBI?)
L4       10153 S (INCLUS? OR SOLUBILI? OR SOLUB? OR INSOLUB? OR DENATUR? OR RE
L5        1536 S (METHOD? OR PROCESS?) (S) L4
L6          61 S L5 (S) E(W)COLI
L7          47 DUP REM L6 (14 DUPLICATES REMOVED)

=> index bioscience medicine
INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE,
    AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS,
    BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB,
    CROPU, DDFB, DDFU, DGENE, DISSABS, ...' ENTERED AT 09:37:59 ON 05 JUL 2005

77 FILES IN THE FILE LIST IN STNINDEX
=> s pro-urokinase or pro(s)urokinase or urokinase or plasminogen(s)activator
L1  QUE PROUROKINASE OR PRO-UROKINASE OR PRO(S) UROKINASE OR UROKINASE OR PLAS
    MINOGEN(S) ACTIVATOR

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F2      34439  SCISEARCH
F3      33402  BIOSIS
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F5      24460  CAPLUS
F6      14920  USPATFULL
F7      13430  PASCAL
F8      12409  TOXCENTER
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F32 1013 PHIN
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F41 332 ADISNEWS
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COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	4.13	4.34

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FILE 'CANCERLIT' ENTERED AT 11:31:39 ON 05 JUL 2005

FILE 'LIFESCI' ENTERED AT 11:31:39 ON 05 JUL 2005

FILE 'BIOTECHDS' ENTERED AT 11:31:39 ON 05 JUL 2005

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The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

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5 FILES SEARCHED...
8 FILES SEARCHED...
L3 35760 (GENE# OR SEQUENCE# OR CLONE# OR POLYNUCLEOTIDE# OR RECOMBI?) (S) L2

=> s (inclus? or solubili? or solub? or insolub? or denatur? or renatur? or chaotroph# or urea# or guanidine# or refold? or dilut? or purif?) (s)l2
9 FILES SEARCHED...
L4 10153 (INCLUS? OR SOLUBILI? OR SOLUB? OR INSOLUB? OR DENATUR? OR RENAT UR? OR CHAOTROPH# OR UREA# OR GUANIDINE# OR REFOLD? OR DILUT? OR PURIF?) (S) L2

=> s (method? or process?) (s) l4
4 FILES SEARCHED...
L5 1536 (METHOD? OR PROCESS?) (S) L4

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PROCESSING COMPLETED FOR L6
L7 47 DUP REM L6 (14 DUPLICATES REMOVED)

=> d ibib abs l7 1-47

L7 ANSWER 1 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-26514 BIOTECHDS
TITLE: Use of pro-urokinase (pro-UK) mutant in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery;
recombinant protein production via plasmid expression in host cell for use in disease therapy and genie therapy
AUTHOR: GUREWICH V; WILLIAMS J N; LIU J; SARMIENTOS P; PAGANI M
PATENT ASSIGNEE: THROMBOLYTIC SCI INC
PATENT INFO: WO 2004093797 4 Nov 2004
APPLICATION INFO: WO 2004-US11840 16 Apr 2004
PRIORITY INFO: US 2003-464003 18 Apr 2003; US 2003-463930 18 Apr 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-775860 [76]
AN 2004-26514 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A ***pro*** - ***urokinase*** (***pro*** -UK) mutant is useful in treating a person with symptoms of stroke or heart attack which comprises determining that the person potentially has had a stroke or heart attack based on observing one or more symptoms of stroke or

heart attack and administering to the person a composition comprising an amount of the ***pro*** -UK mutant effective to lyse any potential blood clot causing the symptoms of stroke or heart attack, is new.

DETAILED DESCRIPTION - A ***pro*** - ***urokinase*** (***pro*** -UK) mutant is useful in treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery. INDEPENDENT CLAIMS are also included for: (1) an intravascular expandable catheter for delivering to a vascular site in a patient an activated, two-chain ***pro*** - ***urokinase*** (tcpro-UK) mutant; (2) an intravascular device for delivering to a vascular site in a patient an activated, two-chain ***pro*** - ***urokinase*** (tcpro-UK) mutant comprising a body and a carrier layer arranged on a surface of the body, where the carrier layer comprises a sustained release agent that slowly releases over time an amount of a tcpro-UK mutant effective to lyse thrombi or emboli in contact with the body; (3) a ***method*** of preparing a ***pro*** - ***urokinase*** (***pro*** -UK) mutant polypeptide; (4) a composition comprising an isolated, single-chain ***pro*** - ***urokinase*** (***pro*** -UK mutant polypeptide, where at least 96% of the protein in the composition is the single-chain ***pro*** -UK mutant polypeptide; (5) a ***purified*** culture of ***E*** ***coli*** type B strain bacteria BL21/DE3 RIL, where bacteria in the culture comprise an expression plasmid encoding a ***pro*** - ***urokinase*** flexible loop mutant polypeptide; and (6) preparing a ***pro*** - ***urokinase*** (***pro*** -UK) mutant polypeptide.

BIOTECHNOLOGY - Preferred Mutant: The new ***pro*** - ***urokinase*** (***pro*** -UK) mutant is an activated, two-chain ***pro*** - ***urokinase*** (tcpro-UK) mutant for clearing a lumen of blood clots, which comprises obtaining a lumen that contains or may contain a blood clot and flowing through the lumen a solution comprising an activated, tcpro-UK mutant for a time sufficient for any blood clots to be dissolved. The solution comprises a concentration of tcpro-UK mutant of 0.05-0.2 mg. The lumen is in a catheter, blood pump or artificial organ. The tcproUK mutant is a low molecular weight tcpro-UK mutant. The new ***pro*** - ***urokinase*** (***pro*** -UK) mutant is useful in treating a person with symptoms of stroke or heart attack which comprises determining that the person potentially has had a stroke or heart attack based on observing one or more symptoms of stroke or heart attack and administering to the person a composition comprising an amount of the ***pro*** -UK mutant effective to lyse any potential blood clot causing the symptoms of stroke or heart attack. The ***pro*** - ***urokinase*** (***pro*** -UK) mutant is useful in lysing occlusive thrombi and emboli in a patient before, during, or after surgery, which comprises administering to the patient within 5 hours before surgery, during surgery, or within 24 hours after surgery, a composition comprising the ***pro*** -UK mutant effective to preferentially lyse any potential occlusive thrombus or embolus compared to hemostatic fibrin in wound sealing clots. The ***pro*** -UK mutant comprises a ***pro*** -UK flexible loop mutant. The ***pro*** -UK mutant comprises the mutation Lys300 to His. The composition is administered more than 3 hours after the onset of symptoms. A bolus of the composition comprising 20-50 mg of the ***pro*** -UK mutant is administered. The ***method*** further comprises obtaining a medical confirmation of an occlusive thrombus in the brain and administering an infusion of the composition at a ***pro*** -UK mutant dosage of dose of 120-200 mg/hour (intravenous) or 50-100 mg/hour (intra-arterial). The composition is administered within 90 minutes of the onset of symptoms. The ***pro*** - ***urokinase*** (***pro*** -UK) mutant is useful in lysing occlusive thrombi and emboli in a patient before, during, or after surgery, which comprises administering to the patient within 5 hours before surgery, during surgery, or within 24 hours after surgery, a composition comprising the ***pro*** -UK mutant effective to preferentially lyse any potential occlusive thrombus or embolus compared to hemostatic fibrin in wound sealing clots. The composition is administered by infusion within three hours before, during or after surgery. The composition is administered by infusion at a ***pro*** -UK mutant dosage of 50 - 200 ml/hour. Preferred Catheter: The intravascular expandable catheter for delivering to a vascular site in a patient an activated, two-chain ***pro*** - ***urokinase*** (tcpro-UK) mutant comprises: (1) a catheter body having proximal and

distal ends; (2) an expandable portion arranged at the distal end of the catheter body; and (3) a carrier layer arranged on a surface of the expandable portion, where the carrier layer comprises an amount of a tcpro-UK mutant effective to lyse thrombi or emboli in contact with the expandable portion. The carrier layer is a hydrogel selected to quickly release effective amounts of the tcpro-UK mutant upon contact with a thrombus or embolus. The amount of the tcpro-UK mutant comprises 0.1-0.5 mg. The carrier layer comprises a lumen containing the tcpro-UK mutant and one or more apertures that are pressed against a thrombus or embolus to allow the thrombus or embolus to protrude into the one or more apertures, thereby contacting the tcpro-UK mutant. The carrier layer comprises activated, two-chain ***pro*** -UK mutant M5. Preferred Device: The device is a stent or suture. Preferred Composition: The composition comprises an isolated, single-chain ***pro*** - ***urokinase*** (***pro*** -UK) mutant polypeptide, where at least 98% of the protein in the composition is the single-chain ***pro*** -UK mutant polypeptide, and an acidic excipient. Preferred ***Method*** : Preparing a ***pro*** - ***urokinase*** (***pro*** -UK) mutant polypeptide comprises: (1) obtaining a nucleic acid molecule that encodes a ***pro*** -UK mutant polypeptide; (2) inserting the nucleic acid molecule into a pET29a expression plasmid comprising a phage T7 promoter and Shine-Dalgarno sequence; (3) transforming ***E*** . ***coli*** type B strain bacteria BL21/DE3 RIL with the expression plasmid; (4) culturing the transformed bacteria for a time and under conditions sufficient to enable the bacteria to express ***pro*** -UK mutant polypeptide; and (5) isolating the ***pro*** -UK mutant polypeptide from the transformed bacteria. The ***pro*** -UK mutant is non-glycosylated and has a molecular weight of about 45,000 daltons. The culturing comprises a two-stage fermentation. The first stage of fermentation comprises adding to a flask a cell culture ***diluted*** in sterile EC1 medium and growing the culture at about 34 to 37 degrees C for at least about 10 hours with agitation to form a seed culture, where the cell culture comprises a glycerol suspension of an LB culture of the transformed bacteria and containing a sufficient amount of kanamycin. The second stage of fermentation comprises: (1) adding the seed culture to a fermenter; (2) maintaining the pH in the fermenter at about 6.8 to 7.2; (3) maintaining the dissolved oxygen concentration in the culture medium at about 35 to 45% of air saturation; (4) maintaining the temperature of fermentation at about 34 to 37 degrees C; and (5) adding to the fermentor a nutrient feeding solution comprising one or more sugars when all glucose initially present in the fermentor at step (1) is consumed following the equation $V = Vo e^{18t}$. V = is volume of feeding solution added (ml/h); Vo = is 1/100 of the starting fermentation medium (ml); and t = is time of fermentation after the start of the feeding phase (hours). The expression plasmid containing the nucleic acid molecule is pET29aUKM5. The ***method*** further comprises preparing two-chain ***pro*** -UK mutant by passing the ***pro*** -UK mutant over plasmin bound to a substrate. The substrate is an agarose-based gel filtration medium. The ***method*** further comprises combining the isolated ***pro*** -UK mutant polypeptide with an acidic excipient. Preparing a ***pro*** - ***urokinase*** (***pro*** -UK) mutant polypeptide comprises: (1) obtaining a transformed bacteria, where the bacteria is an ***E*** . ***coli*** type B strain bacteria BL21/DE3 RIL transformed with a pET29a expression plasmid comprising a phage T7 promoter, a Shine-Dalgarno sequence, and a nucleic acid molecule that encodes a ***pro*** -UK mutant polypeptide; (2) culturing the transformed bacteria for a time and under conditions sufficient to enable the bacteria to express ***pro*** -UK mutant polypeptide; and (3) isolating the ***pro*** -UK mutant polypeptide from the transformed bacteria.

ACTIVITY - Thrombolytic; Cardiant; Cerebroprotective; Vasotropic. No biological data given.

MECHANISM OF ACTION - Gene therapy.

USE - The ***pro*** - ***urokinase*** (***pro*** -UK) mutant is useful in clearing a lumen of blood clots or for lysing occlusive thrombi and emboli for treating a person with symptoms of stroke or heart attack in a patient before, during or after surgery. The composition comprising an aliquot of 20 to 40 mg of a ***pro*** -UK mutant, packaged with directions is useful in administering as a bolus or by infusion to a patient exhibiting symptoms of a stroke or a heart attack. (All claimed.)

ADMINISTRATION - The composition is administered via intravenous route. No biological data given.

EXAMPLE - No relevant examples given. (90 pages)

L7 ANSWER 2 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-20406 BIOTECHDS
TITLE: Producing soluble proteins using transformed genes encoding protein-folding-related factors in a cell-free protein synthesis system, useful in therapeutic, industrial and research purposes;
recombinant protein production via plasmid expression in host cell for use in genomics
AUTHOR: KANG S H; CHOI W J; KIM H J; JUN S Y; LEE K Y
PATENT ASSIGNEE: DREAMBIOGEN CO LTD
PATENT INFO: WO 2004072107 26 Aug 2004
APPLICATION INFO: WO 2004-KR302 13 Feb 2004
PRIORITY INFO: KR 2003-9628 15 Feb 2003; KR 2003-9628 15 Feb 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-616042 [59]
AN 2004-20406 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Producing ***soluble*** protein comprises by cells transformed by genes encoding protein-folding-related factors (PFRF), so that the transformed cells can express enhanced levels of the PFRFs, over-expressing the factors and preparing a cell extract for a cell-free protein synthesis system from the transformed cells, and producing a high level of ***soluble*** protein in the synthesis system containing the extract containing the factors.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a cell-free protein synthesis kit comprising the cell extract containing a substantial amount of folding-related factor as cited above.

BIOTECHNOLOGY - Preferred ***Method*** : The folding-related factor in producing a ***soluble*** protein is one or more in number, and/or is a chaperone that is GroES/GroEL chaperone family or DnaK/DnaJ/GrpE chaperone family. The cell extract is prepared from one or more transformed cells. The protein is alpha-, beta-, gamma-interferon, lipase, erythropoietin, cytokines, interleukins, granulocyte-colony stimulating factor, granulocyte macrophage-colony stimulating factor, transforming growth factors, thrombopoietin, or tissue ***plasminogen*** ***activator*** .

USE - The ***methods*** and compositions of the present invention are useful for producing ***soluble*** proteins applicable to therapeutic, industrial and research purposes, in particular for assaying functions of newly discovered genes.

EXAMPLE - Transformed cells harboring genes of folding-related factors were prepared by standard transformation ***methods*** . More specifically, BL21(DE3) as an ***E*** . ***coli*** strain was used as a host cell and plasmids encoding folding-related factors were obtained. Commercially available vectors were also used instead as a vector for expression of chaperone. GroES/GroEL chaperone family or DnaK/DnaJ/GrpE chaperone family was used as the folding-related factors. The transformants harboring genes of folding-related factors were cultured in LB media containing antibiotics. The expression of T7 RNA polymerase and folding-related factors was induced when the cell density reached 0.6. French press was used at 770 psi to disrupt the cells. The resultant cell extract was centrifuges, and the supernatant collected.(58 pages)

L7 ANSWER 3 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-20939 BIOTECHDS
TITLE: Novel mutant proteinase inhibitor comprising a mutation in an epitope of amino acid sequence of wild-type proteinase inhibitor, useful for screening compounds that affect inhibitory activity of the proteinase inhibitor;
enzyme-inhibitor production for use in drug screening
AUTHOR: LAWRENCE D A; GORLATOVA N; CRANDALL D L
PATENT ASSIGNEE: AMERICAN NAT RED CROSS
PATENT INFO: WO 2003053921 3 Jul 2003
APPLICATION INFO: WO 2002-US22822 18 Jul 2002

PRIORITY INFO: US 2001-305908 18 Jul 2001; US 2001-305908 18 Jul 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-569214 [53]
AN 2003-20939 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Mutant proteinase inhibitor (I) comprising a wild-type proteinase inhibitor amino acid sequence with at least 1 mutation in at least 1 epitope of the amino acid sequence, where the mutation alters the binding of the mutant proteinase inhibitor to an anti-proteinase inhibitor antibody as compared to the binding of the wild-type proteinase inhibitor to the anti-proteinase inhibitor antibody, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a fragment (II) of (I), where the fragment comprises at least one mutation in at least one epitope of the amino acid sequence; (2) a nucleic acid sequence (III) comprises a nucleotide sequence encoding (I) or (II); (3) a nucleic acid construct (IV) comprising (III) operably linked to regulatory control sequences that effect the expression of the nucleic acid sequence; (4) a vector (V) comprising (IV); (5) a host (VI) transformed or transfected with (IV); (6) preparation of (I); and (7) mapping (M1) at least one compound binding site in a metastable protein involves incubating a compound with a mutant metastable protein or its fragment, and separately incubating the compound with a wild-type metastable protein or its fragment, measuring the effect of the compound on a measurable activity of the mutant metastable protein or the fragment and the wild-type metastable protein or the fragment, and comparing the effect of the compound on the metastable activity of the mutant metastable protein or the fragment with the effect of the compound on the metastable activity of the wild-type metastable protein or the fragment.

BIOTECHNOLOGY - Preparation: Producing (I) involves culturing (VI) under conditions, where the nucleic acid sequence is expressed (claimed). Preferred Inhibitor: The anti-proteinase inhibitor antibody has a lower binding affinity to the mutated epitope of the mutant proteinase inhibitor than the anti-proteinase inhibitor antibody has for the wild-type proteinase inhibitor. The anti-proteinase inhibitor antibody affects a measurable activity of the wild-type proteinase inhibitor, the measurable activity being the inhibitory activity of the wild-type proteinase inhibitor against a target proteinase. The anti-proteinase inhibitor antibody does not bind to the mutated epitope of the mutated proteinase inhibitor. (I) is a mutant of wild-type ***plasminogen*** ***activator*** inhibitor (PAI), preferably wild-type PAI-1, and the anti-proteinase inhibitor antibody is an anti-PAI monoclonal antibody e.g., MA-33B8 or 31C9, where the mutant protease inhibitor comprises a substitution at amino acid residues 87, 88, 89, 174, 230, 232, 329, and 331, preferably at least one amino acid substitution at amino acid 87, where the amino acid residue asparagine at position 87 is changed to aspartic acid. The amino acid sequence of wild-type of PAI-1 comprises a substitution at one or more amino acid residues chosen from 1, 2, 13, 82, 87, 88, 89, 92, 95, 110, 115, 127, 151, 174, 196, 202, 212, 218, 223, 230, 241, 283, 300, 305, 308, 329, 331, 323, 339, 351 or 354, preferably at amino acid residues 87, 88, 89, 174, 196, 230, 232, 283, 329 and 331. Most preferably, the mutant comprises at least one amino acid substitution at amino acid 230 (changing glycine to valine) or at amino acid 331 (changing serine to arginine). Preferred ***Method*** : (M1) further involves: when the compound affects the measurable activity of the wild-type stable protein, selecting a compound that has less of an effect on the activity of the mutant metastable protein or the fragment that the effect on the activity of the wild-type stable protein or the fragment. The mutant metastable protein or its fragment retains from 5-100% of its measurable activity in the presence of the compound. The mutant metastable protein or the fragment is prepared by introducing at least one mutation in at least one epitope of the amino acid sequence of the wild-type metastable protein or the fragment, where the mutation alters the binding of the mutant metastable protein or its fragment to an antibody that binds to the wild-type metastable protein or its fragment.

USE - (I) is useful for screening at least one compound that affects the activity of a proteinase inhibitor, which involves incubating a compound with (I) or (II), where the inhibitor or the fragment comprises at least one mutation in at least one epitope of the amino acid sequence, and separately incubating the compound with, a wild-type proteinase

inhibitor or its fragment; measuring the binding of the mutant proteinase inhibitor or the fragment and the wild-type proteinase inhibitor or the fragment with the compound, and comparing the binding of the compound to the mutant proteinase inhibitor or the fragment with the binding of the compound to a wild-type proteinase inhibitor or the fragment. The ***method*** further involves selecting a compound that binds less strongly with the mutant proteinase inhibitor or the fragment as compared to the binding of the compound to the wild-type proteinase inhibitor or the fragment. The compound binds to the mutant proteinase inhibitor or the fragment about 95% or less, most preferably 24% or less than the binding of the compound to the wild-type proteinase inhibitor or fragment. The compound does not bind to the mutant proteinase inhibitor or the fragment but does bind to the wild-type proteinase inhibitor or the fragment. (I) is useful for screening at least one compound that affects the inhibitory activity of a proteinase inhibitor which involves incubating a compound with (I) or (II), and separately incubating the compound with a wild-type proteinase inhibitor or its fragment; measuring the effect of the compound on the inhibitory activity of (I) or (II) and the wild-type proteinase inhibitor on a target proteinase, and comparing the effect of the compound on the inhibitory activity of the mutant proteinase inhibitor or the fragment with the effect of the compound on the inhibitory activity of a wild-type proteinase inhibitor or the fragment. The ***method*** further involves: when the compound affects the inhibitory activity of the wild-type proteinase, selecting a compound that has less of an effect on the inhibitory activity of the mutant proteinase inhibitor or its fragment than the effect on the inhibitory activity of the wild-type proteinase inhibitor or the fragment. The mutant proteinase inhibitor or its fragment retains from 5-100% (preferably 75%) of its inhibitor activity against the target proteinase as measured in the absence of the compound. The compound has substantially no effect on the inhibitory activity of the mutant proteinase inhibitor or the fragment. The measuring step involves adding a target proteinase to the mutant proteinase inhibitor and the compound to form a first mixture and to the wild-type proteinase inhibitor to form a second mixture and incubating the first and second mixtures. The measuring step comprises measuring the target proteinase activity by adding a substrate of the target proteinase and measuring the enzymatic conversion of the substrate. The screening further comprises control treatment comprising, performing a parallel set of the above steps, except that no compound is added in first step. The ***method*** further involves performing a control incubation, where the target proteinase activity is measured without the addition of the mutant proteinase inhibitor or the fragment and the wild-type proteinase inhibitor or the fragment in first step, and then performing a control incubation, where the target proteinase activity is measured without the addition of the mutant proteinase inhibitor or the fragment, the wild-type proteinase inhibitor or the fragment and the compound in first step (all claimed).

EXAMPLE - A library of random ***plasminogen*** ***activator*** inhibitor (PAI)-1 mutants with greater than 2 x 107 independent clones was constructed in the lambda phage expression vector lambdaEXlox by error-prone PCR. Briefly, the entire coding sequence of mature PAI-1 was amplified by four cycles of error-prone PCR. The mutagenized product was gel ***purified*** from the template and re-amplified using standard PCR conditions to produce an amplified pool of randomly mutagenized PAI-1 cDNAs. The PCR product was cleaved with XbaI and EcoRI, ligand to similarly restricted lambdaEXlox arms, and packaged. Lawns of phage-infected Escherichia coli BL21 (DE3) were prepared from exponentially grown cells. To select phage plaques with mutated PAI-1 molecules, protein expression was induced by incubating pregrown phage plaques with overlaid isopropyl-beta-D-thiogalactoside (IPTG) (10 mM) + tissue ***plasminogen*** ***activator*** (tPA) (10 microg/ml) saturated nitrocellulose filters within 1 hour at 37 degreesC. Lifted filters were once washed and incubated with 33B8 mAbs, as a primary antibodies. Filter-bound (PA:PAI-1:33B8 mAbs complexes were detected immunologically with goat anti-mouse immunoglobulins, conjugated to horse radish peroxidase (HRP) in enhanced chemiluminescence (ECL) assay. The dark spots revealed on ECL Hyperfilms indicated plaques expressing PAI-1 molecules capable of binding to the 33B8 mAbs. Then filters were washed, incubated with rabbit anti-PAI-1 pAbs as the primary

and goat anti-rabbit alkaline phosphatase (AP) conjugated antibodies as secondary ones. To select 33B8 binding-negative plaques, the ECL Hyperfilms were overlapped with the original nitrocellulose filters. Plaques expressing 33B8 mAbs binding-negative phenotype were picked up from relevant bacterial plates and run through two sequential rounds of enrichment. Totally, three hundred thousand of lambdaEXlox particles from L5 random mutations of hour PAI-1 library were screened, and from 22 particles expressing active PAI-1 phenotype which were picked up in the first screen only 11 appeared to be positive after following two screens, and thus were isolated. Individual PAI-1 site-directed mutants were constructed by site-directed mutagenesis. After final transformation in ***E*** . ***coli*** XL-10 Gold competent cells with the mutated PAI-1 DNA sequences, several clones of each individual mutant (at least four) were selected and sequenced throughout the entire PAI-1 coding region with 3-5 sequencing primers. Eventually, eight of the site-directed mutants, with the single amino acid replacement in positions 87, 88, 89, 174, 230, 232, 329 and 331 of PAI-1 molecule were constructed and evaluated. The binding of ***purified*** hour PAI-1 mutant proteins and ***E*** . ***coli*** BL21 (DE3) Tuner cell-free extracts (CEs) to specific murine anti-hour PAI-1 33B8 mAbs was analyzed by surface plasmon resonance (SPR) ***method*** . The identified mutants of PAI-1 that did not bind to the 33B8 antibody with high affinity and also were not inactivated by 33B8. These data indicated that both wild-type PAI-1, and the previously identified mutant of PAI-1, 14-1b were both rapidly inactivated by the antibody. In contrast, two of the newly constructed single point mutations. S331R and D89G were not rapidly inactivated by 33B8. The demonstrates that mutations that disrupt binding of 33B8 to PAI-1 also prevent 33B8 for rapidly inactivating the PAI-1.(46 pages)

L7 ANSWER 4 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-09333 BIOTECHDS
TITLE: Producing recombinant protein e.g. two chain-urinary type plasminogen activator, by incubating cell line genetically transfected with cloned precursor cDNA sequence in culture medium containing alkanoic acids;
plasmid pTZA9-mediated CHO cell transformation and cell culture for urokinase production and application in cardiovascular disease therapy
AUTHOR: ARINI A; COPPOLECCHIA R; PAGANI F P; HERBST D; TOGNINI A
PATENT ASSIGNEE: CERBIOS-PHARMA SA
PATENT INFO: ZA 200202136 31 Jul 2002
APPLICATION INFO: ZA 2002-2136 15 Mar 2002
PRIORITY INFO: US 2001-815533 16 Mar 2001; US 2001-815533 16 Mar 2001
DOCUMENT TYPE: Patent
LANGUAGE: Unavailable ZA
OTHER SOURCE: WPI: 2003-184660 [18]
AN 2003-09333 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Producing (M1) a mature recombinant protein into the culture medium of an eukaryotic cell line genetically transfected with a cloned precursor cDNA sequence, comprising incubating the cell line in the cell culture medium, where alkanoic acids, their derivatives or salts have been added for at least 24 hours, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) isolating (M2) recombinant high molecular weight (HMW) and/or low molecular weight (LMW) two chain-urinary type ***plasminogen*** ***activator*** (tc-uPA) from an exhausted culture media of genetically engineered CHO cells using the cell culture supernatant obtained by (M1); (2) recombinant tc-uPA obtainable by (M1); (3) recombinant HMW and LMW tc-uPA product obtainable by (M2); (4) recombinant ***purified*** HMW or LMW tc-uPA obtainable by (M2); and (5) pharmaceutical compositions comprising recombinant ***purified*** HMW or LMW tc-uPA as above, as an active agent.
BIOTECHNOLOGY - Preferred ***Method*** : The cDNA sequence encodes for a protein precursor which encodes for the human Pre-***prourokinase*** . The mature recombinant protein is tc-uPA such as HMW or LMW. The alkanoic acids and/or their salts and/or derivatives are butyric acid, sodium butyrate, sodium propionate, magnesium butyrate, tributyrin or phenyl-butyrate, at a concentration of 0.1-20 mM. The

eukaryotic cell line is mammalian cell line chosen from HEK-293, CV-1, COS, BSC-1, Madin-Darby canine kidney (MDCK), A-431, Chinese Hamster ovary (CHO), Baby Hamster kidney (BHK), and CHO-Messi. The incubation is performed at at most37degreesC for 48-200 hours. The cell culture is serum-free culture medium. For producing recombinant tc-uPA, the cells are cultured at 30-37degreesC, preferably 33-35degreesC for 72-150 hours. The cell viability of CHO cell culture is at least 70%. (M2) comprises an ion-exchange chromatography. The separation of recombinant HMW from LMW tc-uPA comprises acidification of the cell culture supernatant with a weak acid to pH values 5-5.8, optionally adding a non-ionic detergent, contacting the acidified supernatant with a ion-exchange chromatography column at pH values 5.5-6.5, releasing the LMW tc-uPA by adding a buffer solution with a pH of 5.5-6.5, comprising a monovalent ion in concentration of 200-300 mM, releasing the HMW tc-uPA by adding a buffer solution with pH 6-7.5, comprising monovalent ions of 400 nM. The acidified supernatant is additionally filtered. The ***purification*** of recombinant tc-uPA HMW comprises benzamidine chromatography. The ***method*** comprises contacting the released HMW tc-uPA containing buffer solution with a benzamidine column, at pH 6.2-6.8, releasing the tc-uPA HMW with a buffer solution with a pH value comprised 3.8-4.2, further comprising monovalent ions in 300-500 mM, further optionally contacting the released tc-uPA HMW with a gel-filtration column and releasing of the HMW tc-uPA with a low-salt solution buffer at pH 4-7. The ***method*** further comprises contacting the released LMW tc-uPA containing solution with a benzamidine column, at pH 6-8, releasing the tc-uPA LMW with a buffer solution with a pH 3.8-4.2, further comprising monovalent ions in 300-500 mM, further optionally contacting the released tc-uPA LMW with a gel-filtration column and releasing of the LMW tc-uPA with a low-salt solution buffer at pH 4-7.

ACTIVITY - Cardiant; Anticoagulant. No supporting data is given.

MECHANISM OF ACTION - Thrombolytic.

USE - (M1) is useful for producing a mature recombinant protein, especially a recombinant tc-uPA. (M2) is useful for isolating recombinant HMW and/or LMW tc-uPA from an exhausted culture media of genetically engineered CHO cells. The recombinant tc-uPA isolated is useful for treating myocardial infarction and thromboembolytic disorders such as peripheral arterial occlusion (PAOD), catheter clearance, pulmonary embolism and deep venous thrombosis (claimed).

ADVANTAGE - When genetically modified eukaryotic cells are treated for a time of at least 24 hours with alkanoic acids, the conversion of a recombinant precursor protein into the corresponding mature form is very efficient and a mature active protein is accumulated at high amount into the cell culture supernatant.

EXAMPLE - The cDNA sequence encoding for the human pre-***prourokinase*** (pre-proUK) (corresponding to sequence ID D00244 in GenBank) was synthesized from the mRNA of a human kidney cell line (CAKI-1). cDNA was synthesized by AMV reverse transcriptase reaction in the presence of the mRNA mixture, Oligo dT18 and a mixture of the four deoxynucleotides. The mixture of cDNA molecules was specifically amplified by polymerase chain reaction (PCR) with (i) and (ii) primers. The 1296 nucleotides long cDNA sequence encoding the human pre-proUK sequence was obtained and cloned into a pBR322 derived integrative expression vector. The vector contained human pre-proUK cDNA sequence, under the control of the viral SV40 early promoter, the TrpB selection marker for the metabolic selection in Chinese Hamster ovary (CHO)-Messi cells, ampicillin resistance as an antibiotic resistance marker in Escherichia coli and ***E*** . ***coli*** origin of replication for amplification in ***E*** . ***coli*** . The final recombinant expression vector obtained was called pTZA9. The CHO cell line used for the production of recombinant two chain-urinary type ***plasminogen*** ***activator*** (tc-uPA) was the CHO-Messi cell line. Transfection of CHO-Messi cells with pTZA9 was performed. Stable transfected cells were obtained after limiting ***dilution*** in selective CHOMaster medium (without tryptophan), with the addition of serine (0.02 g/l) and indole (0.35 g/l). The cell inoculum was performed in a 2.4 l bioreactor by splitting an exponentially growing cell culture. The ratio volume of inoculum/volume fresh medium was chosen between 1:1 and 1:5, according to the total capacity of the bioreactor and to the culture conditions. The following fermentation parameters were set up for the culture, at 37+/-0.5degreesC, pH of 7.15+/-0.1 and pO2 of 50%+/-20%. When the

cellular density reached values of 2x106 living cells/ml, the cells were separated from the exhausted medium by tangential filtration. Cells were then resuspended in the bioreactor in the same original volume of fresh medium CHOMaster with the addition of sodium butyrate to achieve a final concentration of 1.2 mM. The temperature of the culture was lowered to 34+/-0.5degreesC, and the other fermentation parameters were kept as previously set. The production of active u-PA (tc-uPA high or low molecular weight) was monitored by a chromogenic test on a specific substrate. A progressive increase of the activity was observed up to maximal values as high as 7000 IU/ml, achieved after 4-5 days of fermentation. At this point the cells were harvested and subsequently discarded and the exhausted culture medium, containing tc-uPA, was further ***processed*** for ***purification*** . In the bioreactor a gradual decrease of the cell viability was observed from the first to the fifth day in culture after the addition of sodium butyrate. The minimal cell viability value at 1.2 mM butyrate was observed at the fifth day in culture and was not significantly lower than 70% and was therefore still relatively high. At these values a limited release of lysosomal degradation enzymes into the growth medium was expected. The dramatic increase in the expression of tc-uPA during the production ***process*** , occurred mainly during the last 2/3 days of fermentation after the addition of sodium butyrate. 5'-TAGCGCCGGTACCTCGCCACCATGAGA-3' (i); and 5'-TGGAGATGACTCTAGAGCAAAATGACAACCA-3' (ii).(33 pages)

L7 ANSWER 5 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2002-17927 BIOTECHDS
TITLE: Novel expression vector for expressing mammalian plasminogen derivatives in yeast, has nucleotide sequence coding for catalytic domain of plasminogen and/or coding for kringle domains of plasminogen linked to promoter; vector-mediated gene transfer and expression in Hansenula sp., Candida sp., Torulopsis sp. or Pichia pastoris
AUTHOR: COLLEN D J; NAGAI N; LAROCHE Y
PATENT ASSIGNEE: THROMB-X NV
PATENT INFO: WO 2002050290 27 Jun 2002
APPLICATION INFO: WO 2000-BE217 21 Dec 2000
PRIORITY INFO: GB 2001-16702 9 Jul 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-500632 [53]
AN 2002-17927 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A yeast expression vector (I) comprising a mammalian nucleotide sequence operably linked to a promoter, where the mammalian nucleotide sequence codes for the catalytic domain of ***plasminogen*** and further optionally codes for one or more kringle domains of ***plasminogen*** , its mutants or hybrids, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a yeast cell (II) transfected with (I), and comprising a 2433, 750 or 1047 base pair sequence, given in the specification; and (2) a recombinant mammalian protein (III) obtained by expressing the protein in (II).

BIOTECHNOLOGY - Preferred Vector: The mammalian nucleotide sequence is a human nucleotide sequence and codes for ***plasminogen*** , microplasminogen or miniplasminogen. The nucleotide sequence comprises (S) and is fused to a secretion signal. The promoter is an inducible promoter. (I) is able to stably integrate in the yeast genome, e.g. by homologous recombination. Preferred Cell: (II) belongs to the group of the methylotrophic yeasts. (II) is Hansenula, Pichia, Candida or Torulopsis genera, preferably is Pichia pastoris sp..

ACTIVITY - Cardiant; Thrombolytic; Cerebroprotective. A simple extracorporeal loop thrombosis model in rabbits was used for the quantitative evaluation of the thrombolytic effect of human plasmin and microplasmin. White rabbits were anesthetized and thyroidal uptake of radioiodide was blocked by administration of sodium iodide. A 300 micro-l thrombus was formed around a woolen thread introduced longitudinally in each of two adapted insulin syringes from a mixture of 125I labeled fibrinogen platelet poor rabbit plasma, and 0.07 ml thrombin solution. The clot formed quickly and was allowed to age for 30 minutes at 37 degrees C. Two syringes were inserted in an extracorporeal loop of

silicon tubing between a femoral artery and a marginal ear vein. The blood flow was regulated by a peristaltic pump. Thrombotic extension of the clot was prevented by infusion of heparin and the platelet aggregation inhibitor Ridogrel, 30 minutes before starting infusion of wild-type plasmin or the recombinant microplasmin. The extent of thrombolysis was measured as the difference between the radioactivity introduced in the clot and that recovered in the syringes at the end of the experiment. Local infusion was carried out by using a constant rate infusion pump. The extent of thrombolysis was calculated. Clot lysis with the recombinant microplasmin produced minor alpha2 antiplasmin depletion and fibrinogen breakdown and was associated with minor bleeding time prolongation. Infusion of wild type plasmin resulted in a reduction of 80 % of alpha2-antiplasmin and fibrinogen levels, with minor effect on the bleeding time. Thrombolysis with recombinant microplasmin or wild-type plasmin was not associated with extensive systemic activation of the fibrinolytic system as evidenced by the moderate changes in fibrinogen, alpha2-antiplasmin and bleeding time.

MECHANISM OF ACTION - Mediates fibrin proteolysis.

USE - (II) is useful for expressing a mammalian protein comprising the catalytic domain of ***plasminogen*** and further optionally comprising one or more kringle domains of ***plasminogen*** . The mammalian protein has an 810, 249, or 348 residue amino acid sequence, given in the specification. The ***method*** further comprises activating the expressed protein by using the ***plasminogen*** ***activator*** , preferably staphylokinase or its variant. The protein is then stabilized by treating with a stabilizing agent such as lysine, 6-amino hexanoic acid and tranexamic acid. The stabilizing agent comprises a stabilizing medium, preferably an acid solution or an acid buffer such as a citrate buffer with a pH of 3.1. The protein is then dried by lyophilization. (II) expressed the human microplasminogen at a level of at least 100 mg/l, and miniplasminogen at a level of 3 mg/l. (III) is useful for treating a thromboembolic disease in a mammal. (All claimed). The mammalian protein expressed by (II) is useful for treating focal cerebral ischemic infarction (ischemic stroke) or arterial thrombotic diseases such as peripheral arterial occlusive disease or acute myocardial infarction.

ADVANTAGE - (II) produces pure and stable recombinant mammalian plasmin and its derivatives insufficient amount.

EXAMPLE - A vector was constructed for expression of human miniplasminogen in Pichia pastoris. The pPICZalphaA vector contained a 942 base pair fragment containing the alcohol oxidase 1 (AOX1) promoter that allowed methanol-inducible, high level expression in Pichia and targeted plasmid integration to the AOX1 chromosomal locus, the native transcription termination and polyadenylation signal from the AOX1 gene, an expression cassette conferring zeocin resistance to Escherichia coli and Pichia pastoris, a ColE1 origin of replication for propagation and maintenance of the plasmid in ***E*** . ***coli*** , and unique restriction sites (SacI, PmeI, BstXI). The vector contained the secretion signal of the Saccharomyces cerevisiae alpha-factor prepropeptide, allowing expression of heterologous protein as secreted proteins in the medium. The vector Fmyc-psiPli was used to isolate the region encoding the human microplasminogen protein. The oligonucleotide primers LY-MPG1 (5' GGGGTATCTCTCGAGAAAAGAGCCCCTTCATTTGATTG) and LY-MPG2 (5' GTTTTTGTTCTAGATTAATTATTTCTCATCACTCCCTC) were used. The amplified fragment was directionally cloned into the vector pPICZalphaA. The recipient vector-fragment was transformed into ***E*** . ***coli*** strain TG1, and zeocin resistant clones were selected. Based on restriction analysis, a plasmid clone containing an insert of the expected size was retained for further characterization. Sequence determination of the vector pPICZalpha-MPLG1 confirmed the precise insertion of the microplasminogen coding region fused to the alpha factor mating signal. A pPICZalpha derived secretion vector was constructed for miniplasminogen expression, making use of pPICZalpha-MPLG1 vector. The vector FdTet-SN-miniPlg used to isolate a 500 base pair DNA fragment encoding kringle five and part of the catalytic domain of the miniplasminogen protein. Specific oligonucleotide primers given in the specification were used. The amplified fragment was cloned into the vector pPICZalpha-MPLG1. The recipient vector-fragment was transformed into ***E*** . ***coli*** and zeocin resistant clones were selected. 15 micro-g of the vector pPICZalpha-KMPLG1 was digested with PmeI, and the linear DNA (3

micro-g) was used to transform competent Pichia pastoris X33 cells. Zeocin resistant transformants were selected on YPDSZ plates. At the end of the induction culture, the presence of miniplasminogen in the culture supernatant was estimated. The miniplasminogen in 10-fold ***diluted*** supernatants was incubated with streptokinase for 10 minutes to form an active complex. The generated miniplasmin activity, as determined with the chromogenic substrate S2403 at different times, was compared to the activity of known amounts of a ***purified*** ***plasminogen*** preparation. All tested clones were producing miniplasminogen with yields varying between 3 and 15 mg/l. The two clones X33-KMPLG1 6 and X33-KMPLG1 25, showing the highest miniplasmin activity, were selected for subsequent large scale production.(56 pages)

L7 ANSWER 6 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2002-17141 BIOTECHDS
TITLE: Producing recombinant DNA-derived kringle 2 plus serine protease, comprises using a prokaryotic cell expressing a vector having a DNA coding for a heterologous protein operably linked to a DNA coding for the signal peptide OmpA; vector phagemid-mediated gene transfer and expression in Escherichia coli for use in databases
AUTHOR: WERNER R; GOETZ F; TAYAPIWATANA C; MANOSROI J; MANOSROI A
PATENT ASSIGNEE: BOEHRINGER INGELHEIM INT GMBH
PATENT INFO: WO 2002040696 23 May 2002
APPLICATION INFO: WO 2000-EP12920 14 Nov 2000
PRIORITY INFO: GB 2000-27782 14 Nov 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-471625 [50]
AN 2002-17141 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Producing recombinant DNA-derived heterologous protein in prokaryotic cells, where the heterologous protein is secreted extracellularly as an active and correctly folded protein and the prokaryotic cell contains and expresses a vector comprising the DNA coding for the heterologous protein operably linked to the DNA coding for the signal peptide OmpA or its functional derivative, is new.

BIOTECHNOLOGY - Preferred Cell: The prokaryotic cell contains and expresses a vector comprising the DNA coding for the heterologous protein operably linked to the DNA coding for the signal peptide OmpA which is operably linked the nucleic acid molecule defined the sequence TCTGAGGGAAACAGTGAC, or its functional derivative. The prokaryotic cell is an Escherichia coli. Preferred ***Method*** : The ***method*** comprises amplifying the DNA encoding the heterologous protein by polymerase chain reaction (PCR), ***purifying*** the PCR product, and inserting the PCR product into a vector comprising the DNA coding for OmpA signal peptide and the DNA coding for gpIII in such a way that the PCR product is operably linked upstream to the DNA coding for the OmpA signal sequence and linked downstream to the DNA coding of the gpIII of the vector. A stop codon is inserted between the heterologous protein and gpIII, and the vector is expressed by the prokaryotic cell. The heterologous protein is then ***purified*** . The heterologous protein is a human tissue ***plasminogen*** ***activator*** , or its kringle 2 plus the serine protease (K2S) variant, functional or allelic variant, subunit, chemical derivative, fusion protein, or glycosylation variant. The vector is a phagemid vector comprising the DNA coding for OmpA signal peptide and the DNA coding for gpIII. The vector is preferably the pComb3HSS phagemid. The DNA sequence of OmpA comprises or consists of the sequence: ATGAAAAAGACAGCTATCGCGATTGCAGTGGCACTGGCTGGTTTCGC TACCGTGGCCCAGGCGGCC. The DNA of the heterologous protein is preceded by a lac promoter and/or ribosomal binding site.

USE - The ***method*** is useful for commercial large-scale production of heterologous proteins, e.g. K2S, in prokaryotic cells, and is generally applicable in the expression of several different proteins and polypeptides which do not require mammalian glycosylation in prokaryotic host cells. The ***method*** may also be used to obtain DNA sequences of a protein of interest to be expressed from databases and cloned for use.

ADVANTAGE - Unlike previous ***methods*** , the new ***method*** is commercially applicable for large-scale production of

heterologous proteins, and is more cost effective.

EXAMPLE - The kringle 2 plus the serine protease (K2S) portion of tissue ***plasminogen*** ***activator*** (tPA) was amplified from vector p51-3 using primers SK2/174 and ASSP. The amplified 1110 base pair (bp) product was demonstrated by agarose gel electrophoresis and was inserted into pComb3HSS phagemid by double Sfi I cleavage sites on 5' and 3' ends in the correct reading frame. A new vector, pComb3H-K2S, harboring the K2S was generated. In this vector, K2S is flanked upstream by the OmpA signal sequence and downstream by gp3. The correct insertion was verified by restriction analysis with Sfi I, PCR analysis and DNA sequencing. A stop codon was generated between K2S and gp3 in pComb3H-K2S with the help of mutagenic primers, MSTPA and MASTPA. The cycle amplification mixture was digested with Dpn I to degrade the old dam methylated pComb3H-K2S template. After transforming of ***E*** . ***coli*** XL-1 Blue with MpComb3H-K2S, a transformant XM(K2S) was selected. K2S expression in Escherichia coli XM(K2S) was induced by isopropylthiogalactopyranoside (IPTG). Recombinant K2S was detected by using enzyme linked immunosorbant assay (ELISA) both in the periplasmic space and in the culture supernatant. The amount of the heterologous protein in each preparation was determined by sandwich ELISA and related to the standard tPA. Partially ***purified*** K2S from culture supernatant of ***E*** . ***coli*** XM(K2S) revealed a molecular mass of 39 kDA by using sheep anti-tPA antibodies. The negative control, partially ***purified*** culture supernatant of non-transformed ***E*** . ***coli*** XL1-Blue, contained no reactive band with a similar size. (52 pages)

L7 ANSWER 7 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2002-19570 BIOTECHDS
TITLE: Producing active, correctly folded recombinant tissue plasminogen activator, Kringle 2 serine protease in prokaryotic cells by expressing the protein-encoding DNA operably linked to DNA coding for signal peptide OmpA; recombinant plasminogen-activator production for use in stroke, blood-clotting disorder, pulmonary embolism, etc., therapy
AUTHOR: WERNER R; GOETZ F; TAYAPIWATANA C; MANOSROI J; MANOSROI A
PATENT ASSIGNEE: BOEHRINGER INGELHEIM INT GMBH
PATENT INFO: WO 2002040650 23 May 2002
APPLICATION INFO: WO 2000-EP12857 14 Nov 2000
PRIORITY INFO: GB 2000-27779 14 Nov 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-519376 [55]
AN 2002-19570 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Producing (M1) extracellularly secreted, active, correctly folded, recombinant tissue ***plasminogen*** ***activator*** (tPA) (I), Kringle 2 serine protease molecule (K2S) (II), or their variants (Ia,Ib) in prokaryotic cells (C1) by using a (C1) containing and expressing vector comprising DNA encoding (I,II,Ia or Ib) operably linked to DNA coding for signal peptide OmpA or its functional derivative, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a DNA molecule (III) coding for the OmpA protein or its functional derivative, operably linked to a DNA molecule coding for a polypeptide containing the Kringle 2 domain and the serine protease domain of tPA; (2) a fusion protein (IV) of OmpA and K2S, comprising a fully defined sequence of 377 amino acids (S8) as given in the specification, or its fragment, functional variant, allelic variant, a subunit, a chemical derivative or a glycosylation variant; (3) a K2S protein (V) comprising a fully defined sequence of SEGN (S9) or its variant, fragment, functional variant, allelic variant, subunit, chemical derivative, fusion protein or glycosylation variant; (4) a vector (VI) containing (III); (5) a vector pComb3HSS (VII) containing (III), where the expression of the gp III protein is suppressed or inhibited by deleting the DNA molecule encoding the gp III protein or by a stop codon between the gene coding for a polypeptide containing the Kringle 2 domain and the serine protease domain of tissue ***plasminogen*** ***activator*** protein and the gp III gene; and (6) a prokaryotic host

cell (VIII) comprising (III), (VI) or (VII).

WIDER DISCLOSURE - Pharmaceutical compositions comprising the K2S molecule produced by (M1), are also disclosed.

BIOTECHNOLOGY - Preferred ***Method*** : In (M1), the (C1) contains and expresses a vector comprising the DNA coding for (I), (II), (Ia) or (Ib) operably linked to the DNA coding for the signal peptide OmpA which is operably linked to the nucleic acid molecule defined by the sequence (S1) or a its functional derivative. (M1) preferably involves: (a) amplifying the DNA encoding (I), (II), (Ia) or (Ib) by plymerase chain reaction (PCR); (b) ***purifying*** the PCR product; (c) inserting the PCR product into a vector for gpIII in such a way that the PCR product is operably linked upstream to the DNA coding for the OmpA signal sequence and linked downstream to the DNA coding for gpIII is the vector; (d) inserting a stop codon between (I), (II), (Ia) or (Ib) and gp III; (e) expressing the vector in the prokaryotic cell; and (f) ***purifying*** (I), (II), (Ia) or (Ib). Preferably, the vector is a phagemid vector comprising the DNA coding for the OmpA signal peptide and the DNA coding for gpIII. Most preferably, the vector is a pComb3HSS phagemid. The DNA sequence of OmpA linked upstream to K2S comprises a fully defined sequence of 1128 nucleotides (S2) as given in specification or its functional variant or degenerate variant. The DNA sequence of OmpA comprises or consists of the nucleotide sequence (S3). Preferably, the DNA encoding (I), (II), (Ia) or (Ib), is preceded by a lac promoter and/or a ribosomal binding site. The DNA coding for (I), (II), (Ia) or (Ib) is any one of DNA molecules coding for at least 90% of the amino acids 87-527, 174-527, 180-527 or 220-527 of the human tPA protein. The K2S DNA comprises of a fully defined sequence of 1065 nucleotides (S4) as given in specification or its functional variant or degenerate variant. Preferably, the K2S DNA consists of (S4). Preferred DNA Molecule: (III) preferably comprises a fully defined sequence of 1128 nucleotides (S5) as given in specification or its functional or degenerate variant. Preferably (III) consists of (S5). The DNA molecule coding for a polypeptide containing the Kringle 2 domain and the serine protease domain of tPA preferably codes for at least 90% of the amino acids 87-527, 174-527, 180-527 or 220-527 of human tPA. The DNA molecule hybridizes under stringent conditions to a fully defined sequence of 1065 nucleotides (S7) as given in specification, and preferably consists of (S7). The DNA molecule coding for the OmpA protein or its functional derivative hybridizes under stringent conditions to (S6) Preferably, the DNA molecule consists of (S6). Preferred Protein: (IV) preferably consists of (S8). (V) comprises a sequence of SEGNSD, or its variant, fragment, functional variant, allelic variant, a subunit, a chemical derivative or a glycosylation variant. Most preferably, (V) comprises a fully defined sequence of 354 amino acids (S11) as given in the specification, or its variant, fragment, functional variant, allelic variant, a subunit, a chemical derivative or a glycosylation variant. Most preferably, (V) consists of the sequence of (S11). Preferred Vector: (VI) comprises the DNA sequence preceded by a lac promoter and a ribosomal binding site. Preferred Host Cell: (VIII) is an Escherichia coli host cell. TCTGAGGGAAACAGTGAC (S1) ATGAAAAAGACAGCTATCGCGATTGCAGTGGCA CTGGCTGGTTTCGCTACCGTGGCCCAGGCGGCGGCC (S3) ATGAAAAAGACAGCTATCGCGATTGCAGTGG CACTGGCTGGTTTCGCTACCGTGGCCCAGG CGGCC (S6)

ACTIVITY - Cerebroprotective; Cardiant; Thrombolytic. No biological data is given.

MECHANISM OF ACTION - Mediator of fibrin formation and clot dissolution.

USE - M1 is useful for producing recombinant DNA-derived tissue ***plasminogen*** ***activator*** (tPA), Kringle 2 serine protease molecule (K2S), or variants of tPA or K2S molecule in a prokaryotic cell such as Escherichia coli. (III), (VI), (VII) or (VIII) are used in the ***method*** for producing a polypeptide with the activity of tPA protein. Preferably, the molecules are useful in (M1) (all claimed). The DNA molecules, vectors or host cells are useful for producing a polypeptide having the activity of tissue ***plasminogen*** ***activator*** . Recombinant DNA-derived polypeptides from (M1) are useful for manufacturing a medicament for treating stroke, cardiac infarction, acute myocardial infarction, pulmonary embolism, any artery occlusion such as coronary artery occlusion, intracranial artery occlusion (e.g., arteries supplying the brain), peripherally occluded arteries, deep vein thrombosis, or related diseases associated with

unwanted blood clotting.

ADMINISTRATION - The therapeutic substances are administered by intravenous routes e.g. as a single bolus for 5-10 seconds intravenously. No dosage details are given.

ADVANTAGE - The use of the signal peptide OmpA alone and/or in combination with the N-terminal amino acids SEGN (S9)/SEGNSD (S10) translocate the recombinant DNA-derived tPA, tPA variant, K2S molecule or K2S variant to the outer surface and facilitates the release of the functional and active molecule into the culture medium to a greater extent than any other known ***method*** . Before crossing the outer membrane, the recombinant DNA-derived protein is correctly folded, the signal peptide is cleaved off to produce a mature molecule and the efficiency of signal peptide removal is very high and leads to correct folding of the recombinant DNA-derived protein.

EXAMPLE - To amplify a specific part of tissue ***plasminogen*** ***activator*** (tPA) gene, a pair of primers sK2/174 and ASSP (P1 and P2 respectively) were synthesized. These primers were designed based on the human tPA gene retrieved from NCBI databases (g137119). They were synthesized with SfiI end cloning sites in such a way that the reading frame, from the ATG of the gpIII gene in phagemid vector, pComb3HSS, will be maintained throughout the inserted sequence. Another primer set for site-directed mutagenesis was designed to anneal at the sequence situated between the K2S gene and gene III in pComb3H-K2S. The sequence of primers with mutation bases for generating a new stop codon were MSTPA and MASTPA (P3 and P4 respectively). One microg sK2/174 and ASSP primers together with 50 ng of p51-3 template and amplification of the kringle 2 plus the serine protease portion of tPA (Ser174) in kringle 2 domain to Pro527) in the serine protease was performed. The amplified product of 1110 bp was subsequently ***purified*** and was inserted into pComb3HSS phagemid by double SfiI cleavage sites on 5' and 3' ends in the correct reading frame. Thus a new vector, pComb3H-K2S, harboring the Kringle 2 serine protease molecule (K2S) was generated. In this vector K2S was flanked upstream by the OmpA signal sequence and downstream by gp3. The correct insertion of K2S was verified both by restriction analysis with SfiI, polymerase chain reaction-analysis and DNA sequencing. VCSM13 filamentous phage was used to infect pComb3H-K2S transformed Escherichia coli XL-1 Blue, X(K2S). VCSM13 was propagated and incorporated the K2S-gp3 fusion protein during the viral packaging ***processes*** . The harvested recombinant phage (K2S-phi) gave a concentration of 5.4x1011 colony forming units (cfu)/ml determined by reinfecting ***E*** . ***coli*** XL-1 Blue with PEG-precipitated phages. These recombinant phage particles were verified for the expression of r-K2S by sandwich enzyme linked immunosorbent assay (ELISA). The phage-bound heterologous K2S protein was recognized by the monoclonal anti-kringle 2 antibody by using sheep anti-tPA conjugated horseradish peroxidase (HRP) antibody detection system. The amount of K2S detectable on 1012 phage particles was equal to 336 ng of protein in relation to the standard melanoma tPA. In order to corroborate that K2S-gp3 fusion protein was associated with phage particles, sheep, anti-tPA conjugated HRP antibody was substituted by sheep anti-M13 antibody conjugated HRP. This immuno-reaction exhibited an absorbance of 1.89+/-0.07. In contrast, if the capture antibody was sheep anti-M13 antibody, extremely low K2S was observed with sheep anti-tPA antibody conjugated HRP, the absorbance was only 0.17+/--0.01. This suggested that only a minority of ***purified*** phage particles carried K2S-gp3 fusion protein. VCSM13 prepared from non-transformed XL-1 Blue was used as a negative control. A stop codon was generated between K2S and gp3 in pComb3H-K2S with the aid of the mutagenic primers (MSTPA and MASTPA). In order to enrich the newly synthesized and mutated MpComb3H-K2S, the cycle amplification mixture was thoroughly digested with DpnI to degrade the old dam methylated pComb3H-K2S template. After transforming of ***E*** . ***coli*** XL-1 Blue with MpComb3H-K2S, a transformant XM(K2S) was selected for further study. As a consequence of bp substitution, one SfiI cleavage site close to the 3' and of K2S gene was lost after site-directed mutagenesis. A linear version of SfiI cleaved MpComb3H-K2S was observed at 4319 bp without the appearance of inserted K2S gene fragment. Thus, the K2S gene encoded by MpComb3H-K2S was expressed in non-gp3 fusion form in XM(K2S). K2S expression in XM(K2S) was induced by isopropylthiogalactoside (IPTG). r-K2S was detectable by using ELISA both in the periplasmic space and in the culture supernatant. The amount of the heterologous protein in each

preparation was determined by sandwich ELISA and related to the standard tPA. From 100 ml of the bacterial culture in shaker flask with the O.D (600 nm) of 50, the periplasmic fraction yielded 1.38 microg of r-K2S (approximately 32%) whereas 2.96 microg of r-K2S (approximately 68%) was obtained in the ammonium precipitated culture supernatant. Sandwich ELISA was used to verify the PEG precipitated phage from VCSM13 infected XM(K2S) No r-K2S captured by monoclonal anti-kringle 2 antibody was detected by anti-M13 conjugated HRP, indicating that K2S was not presented on the phage particles if gp3 was missing. 5' GAGGAGGAGGTGGCCCAGGCGGCCTCTGAGGGAAACAGTGAC 3' (P1) 5' GAGGAGGAGCTGGCCGGCCTGGCCCGGTCGCATGTTGTCACG 3' (P2) 5' ACATGCGACCGTGACAGGCCGGCCAG 3' (P3) 5' CTGGCCGGCCTGTCACGGTCGTCACGGTCGCATGT 3' (P4)(80 pages)

L7 ANSWER 8 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2002-17112 BIOTECHDS
TITLE: New protein or peptide comprising epitope of plasminogen activator inhibitor-1, useful for screening substance for treating thromboembolic and cardiovascular diseases, cancer or related diseases;
plasmid-mediated recombinant protein gene transfer and expression in Escherichia coli for disease therapy
AUTHOR: DECLERCK P; STASSEN J; BIJNENS A; GILS A; NGO T H
PATENT ASSIGNEE: LEUVEN RES and DEV
PATENT INFO: WO 2002034776 2 May 2002
APPLICATION INFO: WO 2000-BE186 26 Oct 2000
PRIORITY INFO: DE 2001-1010235 2 Mar 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-463353 [49]
AN 2002-17112 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A protein or peptide (I) comprising the epitope of ***plasminogen*** ***activator*** inhibitor-1 (PAI-1) or its fragment or functional derivative, is new.
DETAILED DESCRIPTION - A protein or peptide (I) comprising the epitope of ***plasminogen*** ***activator*** inhibitor-1 (PAI-1) having the amino acids His-Arg-Arg, Glu-Lys-Glu, Glu-Glu-Ile-Ile-Met-Asp-Arg or Glu-Asp-Arg, or their fragment or functional derivative, is new. INDEPENDENT CLAIMS are also included for the following: (1) a protein or peptide (II) comprising two or more of the epitopes of (I) or its functional derivative; (2) nucleic acid (III) encoding (I) or (II); (3) an antibody (IV) specific for (I) or (II); (4) screening (M) for substances capable of inhibiting PAI-1 by incubating (I) or (II), or entire PAI-1 with a test compound in a suited buffer, tissue-type ***plasminogen*** ***activator*** (tPA) or its fragment and a monoclonal antibody specific for (I) or (II), or PAI-1 and a monoclonal antibody specific for tPA that are detectably labeled with a reporter, the unbound antibodies are washed out, the amount of reporter is measured, and the value obtained is compared to the value obtained in the absence of the test compound; (5) an antibody (V) or its fragment for use in (M) binds specifically to (I) or (II), or PAI-1, or the protein or peptide having formed a complex with tPA; (6) an antibody (VI) or its fragment for use in (M) binds specifically to tPA, tPA having formed a complex with PAI-1 or (I) or (II); (7) use of an antibody specific for (I) or specific to a different epitope of PAI-1 in (M); (8) a substance (VII) identifiable by (M) is capable of specifically inhibiting PAI-1; (9) an Fv fragment, Fab fragment, Fab' fragment, or F(ab')2 fragment of (VII); and (10) pharmaceutical composition comprising (VII).
WIDER DISCLOSURE - Also disclosed are: (1) a protein or peptide comprising an epitope of PAI-1 comprising amino acids 1-26 of PAI-1; and (2) a suitable cell or cell line transformed with nucleic acid constructs to express (I) or (II).
BIOTECHNOLOGY - Preferred Peptide: (I) preferably peptide consist of the amino acids His-Arg-Arg, Glu-Lys-Glu, Glu-Glu-Ile-Ile-Met-Asp-Arg or Glu-Asp-Arg. Preferred Antibody: (IV) is induced by immunization with (I) or (II).Preferred ***Method*** : In (M), (I), or the entire PAI-1 is labeled to differentiate binding of (I), or PAI-1 to tPA from non-binding or reduced binding. In (M), (V) or (VI) is labeled with Europium kryptate or XL665, respectively. (M) is high throughput screening (HTS) or a ultra

high throughput screening (UHTS). Preferred Substance: (VII) is an antibody preferably a monoclonal antibody having an affinity for (I). The monoclonal antibody is preferably a human monoclonal antibody which is more preferably chimeric humanized monoclonal antibody. The constant region of chimeric monoclonal antibody is human, and also comprises parts of variable region of human origin. The variable region of human origin comprises the conserved or framework regions of the antigen-binding domain.

ACTIVITY - Cytostatic; Vasotropic; Cerebroprotective; Antidiabetic; Neuroprotective; Antiinflammatory; Cardiant.

MECHANISM OF ACTION - Antagonist or modulator of PAI-1. No supporting data is given in the source material.

USE - (I) is useful for identifying an inhibitor of PAI-1. (M) is useful for screening a substance capable of inhibiting PAI-1 (VII) is useful in the manufacture of a medicament for treating thromboembolic and cardiovascular diseases, preferably stroke, or in the treatment of cancer or related diseases (all claimed), the diseases include ischemia, ischemic and hemorrhagic stroke, global cerebral ischemia with heart stoppage, post-ischemic neurocytotoxic conditions, hypoglycemia, diabetic polyneuropathy, hypoxia, anoxia, brain edema, brain pressure (elevated intracranial pressure), hypertonia, hypotonia, cardiac infarction, breast cancer, prostate cancer, bladder cancer, liver cancer and cancer of the gastrointestinal tract.

EXAMPLE - ***Plasminogen*** ***activator*** .inhibitor-1 (PAI-1) mutants were created using QuickChange site-directed mutagenesis kit. pIGE20-PAI-1-wild-type (wt) and pIGE20-PAI-1-stab (Vleugels, N., Gils, A., Mannaerts, S., Knockaert, I., and Declerck, P.J. (1998) Fibrinolysis and Proteolysis 12, 277-282) were used as templates to introduce mutation in PAI-1-wt and PAI-1-stab, respectively. After DNA ***denaturation*** polymerase chain reaction cycles were performed and the DNA was subjected to a DpnI digestion prior to transformation of DH1lambda Escherichia coli. For all mutants, large-scale DNA preparations were made and the PAI-1 encoding region was sequenced entirely. PAI-1-wt and PAI mutants were expressed in MC1061 ***E*** . ***coli*** cells which were cotransformed with pAcI and either pIGE20-PAI-1-wt or one of the pIGE20-PAI-1 mutant constructs. Clonal isolates were grown and PAI-1 expression was induced and the cells were harvested and disrupted. The cell lysate was cleared by ultracentrifugation and the PAI-1 containing supernatant was collected and ***purified*** . Then PAI-1 containing elution fraction were examined for their inhibitory activity towards tissue-type ***plasminogen*** ***activator*** (t-PA) by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). PAI-1 neutralization assay was carried out in which the residual PAI-1 activity was quantitated after pre-incubation of human PAI-1 with monoclonal antibodies such as MA-44E4, MA-42A2F6 and MA-56A7C10 and the activity was measured by recording the absorbance change at 405 nm. 100% PAI-1 activity was defined as the PAI-1 activity observed in the absence of monoclonal antibody. The percentage inhibition by the monoclonal antibody was then calculated from the residual PAI-1 activity measured in the presence of the monoclonal antibody. In a PAI-1 neutralization assay, MA-44E4, MA-42A2F6 and MA-56A7C10 inhibited PAI-1 activity in a dose-dependent manner revealed that MA-56A7C10 was the most potent inhibitor (i.e., 80 +/- 5% inhibition using a two-fold molar excess of MA versus 21 +/- 6% and 15 +/- 6% for MA-42A2F6 and MA-44E4, respectively). MA-44E4 binds human PAI-1 with a KA of 4 x 10 to the power of 8M-1, but lacks affinity for porcine, murine and rat PAI-1. Alignment of the sequence of human PAI-1 between residue 81 and 187, the region previously suggested to harbor the major interaction sites for MA-44E4, with the corresponding segment of the latter three species, revealed the presence of only two charged residues, i.e., Asp(181) and Arg(186), that were not conserved in any of the other species, thus one or both of these residues play an essential role in the interaction with the human PAI-1 and MA-44E4. MA-44E4 exhibited an affinity of 4 x 10 to the power of 8 M-1 towards PAI-1-wt. The affinity of PAI-1-D181A (mutant) was nearly identical to that for PAI-1-wt indicated that this residue was not involved in the interaction with MA-44E4. In contrast, MA-44E4 did not bind to PAI-1-H185A-R186A-R187A. This demonstrated that the affinity of MA-44E4 was completely lost by any combinations of two adjacent alanine mutations between residues 185 and 187. Further the single mutation of Arg(186) resulted in a 72-fold decreased binding of MA-44E4, whereas the

single mutation at positions 185 and 187 has a less pronounced effect on the affinity of MA-44E4. (40 pages)

L7 ANSWER 9 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2002-13525 BIOTECHDS
TITLE: Identifying targeting peptides useful for treating e.g. diabetes mellitus, inflammatory diseases, cancer, or autoimmune diseases, comprises exposing a sample to a phage display library and recovering phage bound to the sample; adeno-associated virus vector-mediated recombinant protein gene transfer and expression in host cell, Fab and humanized antibody for use in prostate cancer, Hodgkin disease, diabetes mellitus, inflammatory disease, arthritis, atherosclerosis, autoimmune disease, bacterium infection, virus infection, cardiovascular disease and neurodegenerative disease diagnosis, therapy and gene therapy
AUTHOR: ARAP W; PASQUALINI R
PATENT ASSIGNEE: UNIV TEXAS SYSTEM
PATENT INFO: WO 2002020722 14 Mar 2002
APPLICATION INFO: WO 2000-US27702 8 Sep 2000
PRIORITY INFO: US 2001-765101 17 Jan 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-383050 [41]
AN 2002-13525 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Identifying targeting peptides comprises exposing a sample from an organ, tissue or cell type of interest, to a phage display library and recovering phage bound to the sample (the phage expresses targeting peptides), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a ***method*** (I) of detecting the presence of a target for a targeting peptide; (2) an antibody ***purified*** by the ***method*** of (I); (3) a peptide prepared by the ***method*** (I); (4) a ***method*** comprising attaching an antibody or the peptide to an agent; (5) a ***method*** for identifying an endogenous receptor or ligand; (6) a ***method*** of identifying a virus associated with a disease state; (7) a gene therapy vector (II) comprising: (a) a bacteriophage, which expresses a targeting peptide as part of a surface protein; (b) a gene encoding a therapeutic protein or an anti-sense RNA, and is incorporated into the phage genome; and (c) cis-elements of an adeno-associated virus (AAV), incorporated into the phage genome; (8) a ***method*** of treating a disease by administering a gene therapy vector of (II); (9) an isolated peptide (III) of 100 amino acids or less in size, comprising at least 3 contiguous amino acids of a sequence selected from 225 specified sequences (not defined in the specification); (10) a fusion protein comprising at least 3 contiguous amino acids of the 225 specified amino acid sequences (not defined in the specification); (11) a composition comprising the isolated peptide of (III) in a pharmaceutical carrier; (12) a kit comprising the isolated peptide of (III) and a control peptide, each in a container; (13) an antibody or antibody fragment that selectively binds to an isolated peptide comprising at least 3 contiguous amino acids from the 225 specified amino acid sequences (not defined in the specification); (14) ***methods*** of diagnosing or treating prostate cancer or Hodgkin's disease; (15) a ***method*** of targeting delivery to a prostate cancer or Hodgkin's disease; (16) an antibody or antibody fragment (Fab) (IV) that binds to an isolated protein or peptide comprising a sequence selected from 9 amino acid sequences (not defined in the specification); (17) a gene therapy vector expressing a targeting peptide sequence as part of a surface protein, where the targeting peptide comprises at least 3 contiguous amino acids of the 225 specified amino acid sequences (not defined in the specification); (18) a targeting peptide against human antibodies from an individual with Hodgkin's disease comprising at least 3 contiguous amino acids of the peptide from (IV); (19) a ***method*** for identifying an endogenous homolog for the isolated peptide of (III); (20) an isolated nucleic acid (IV) of 300 nucleotides or less in size, encoding a peptide of (III); (21) a vector comprising the isolated nucleic acid of (IV);

(22) a ***method*** of treating a disease state; (23) a ***method*** of identifying targeting peptides against antibodies from an individual with a disease state; (24) a ***method*** of obtaining antibodies against one or more targeting peptides; and (25) a ***method*** of blocking metastasis.

BIOTECHNOLOGY - Preferred ***Methods*** : In identifying targeting peptides, the phage are recovered by infecting pilus positive bacteria, or by amplifying phage inserts, ligating the amplified inserts to phage DNA, and producing phage from the ligated DNA. The sample is a thin section of an organ or tissue. The bound phage is recovered by Positioning and Ablation with Laser Microbeams (PALMS), or by Biopanning and Rapid Analysis of Selective Interactive Ligands (BRASIL). The ***method*** further comprises: (a) preselecting the phage library against a first organ, tissue or cell type; (b) removing phage that bind to the first organ, tissue or cell type; and (c) selecting the remaining phage against a second organ, tissue or cell type. The first cell type is quiescent and the second cell type has been stimulated with a hormone, growth factor, cytokine, chemokine, neurotransmitter, angiogenic agent, pro-apoptosis agent, anti-apoptosis agent, anti-angiogenic agent, phorbol ester, protein or peptide. Detecting the presence of a target for a targeting peptide comprises exposing a sample of a tissue, organ or cell type consisting of thin section or biopsy sample, to phage expressing one or more targeting peptide sequences, and detecting the presence of phage bound to the sample. The presence of the target indicates a disease, or that the tissue, organ or cell type will respond to a therapy which involves administration of a targeting peptide attached to a therapeutic agent. The therapy is directed against the target, which can be a peptide, protein, glycoprotein, lipoprotein, epitope, lipid, carbohydrate, multi-molecular structure, a specific conformation of one or more molecules or a morphoanatomic entity. The phage library is prescreened against antibodies from a normal subject. The sample comprises antibodies from a subject with a disease such as cancer, metastatic cancer, Hodgkin's disease, ovarian cancer or prostate cancer, and may consist ascites, blood, serum, lymphatic flui d, spleen, tumor or lymph node tissue. The antibodies have been ***purified*** from the sample. The ***method*** further comprises obtaining at least one targeting peptide that binds to antibodies from a cancer subject, producing a vaccine comprising the peptide, immunizing a subject with the vaccine, and activating T cells in vitro with the vaccine. The ***method*** further includes ***purifying*** antibodies that bind to the targeting peptides. The library expresses targeting peptides prepared from antibody sequences. The ***method*** also comprises obtaining a sample of spleen from a subject, obtaining mRNA from the sample, amplifying antibody mRNA sequences to form double-stranded cDNAs encoding antibody peptides, and inserting the cDNAs into a phage display library. The subject is immunized with at least one antigen before collecting the spleen sample. The antibodies that bind to targeting peptides, are attached to an agent selected from a drug, a chemotherapeutic agent, a radioisotope, a pro-apoptosis agent, an anti-angiogenic agent, an enzyme, a hormone, a cytokine, a growth factor, a cytotoxic agent, a peptide, a protein, an antibiotic, an antibody, a Fab fragment of an antibody, an imaging agent, an antigen, a survival factor, an anti-apoptotic agent, a hormone antagonist, a virus, a bacteriophage, a bacterium, a liposome, a microparticle, a magnetic bead, a microdevice, a yeast cell, a mammalian cell, a cell and an expression vector. The ***method*** further comprises administering the antibody to a subject and detecting the presence of an antigen, reacting the antibody and agent with a tissue sample, treating a subject with a disease, and obtaining an image of the subject by detecting the presence of a ligand or receptor for the peptide. Identifying an endogenous receptor or ligand comprises exposing a sample from an organ, tissue or cell type to a phage display library, obtaining phage that bind to the sample, sequencing the phage inserts, and identifying an endogenous receptor or ligand homologous with at least one phage insert. The ***method*** further comprises preparing antibodies against at least one phage insert, and using the antibodies to ***purify*** an endogenous receptor or ligand. Identifying a virus associated with a disease state comprises: (a) pre-clearing a phage display library on immunoglobulins from a subject without a disease; (b) screening the pre-cleared library on immunoglobulins from a subject with a disease

state, such as Hodgkin's disease; (c) recovering phage that bind to immunoglobulins from the subject with the disease state; (d) determining the amino acid sequences of targeting peptides displayed on the collected phage; and (e) identifying one or more viruses encoding proteins homologous with the targeting peptides. Diagnosing prostate cancer or Hodgkin's disease comprises obtaining an antibody or antibody fragment that binds to an isolated peptide comprising at least three contiguous amino acids selected from 31 specified amino acid sequences (not given in the specification), contacting a sample from a subject with the antibody or antibody fragment, and detecting binding of the antibody or antibody fragment to the sample. The antibody or antibody fragment binds to an isolated peptide having a specific amino acid sequence, or to grp78 (glucose regulated protein of 78,000 molecular weight). Alternatively, diagnosis comprises obtaining an isolated protein or peptide of an amino acid sequence selected any of the 31 amino acid sequences, contacting the protein or peptide with an antibody-containing sample from a patient, and detecting binding of an antibody to the protein or peptide. The patient's likelihood of three-year survival is also determined. The sample comprises blood, serum, ascites, lymphatic fluid, spleen, lymph node or prostate tissue, or antibodies from a tumor, a tissue, a peluran effusion or spinal fluid. Treatment of prostate cancer or Hodgkin's disease comprises: (a) obtaining an antibody or antibody fragment that binds to an isolated peptide comprising at least three contiguous amino acids selected from 31 specified amino acid sequences (not given in the specification); (b) contacting a sample from a subject with the antibody or antibody fragment; (c) detecting binding of the antibody or antibody fragment to the sample; (d) preparing a vaccine from the isolated peptide; and (e) immunizing a subject with the vaccine. The ***method*** further comprises stimulating immune system cells in vitro with the vaccine. Targeting delivery to a prostate cancer comprises obtaining an antibody or antibody fragment that binds to an isolated peptide comprising at least three contiguous amino acids selected from any of 31 amino acid sequences (not given in the specification), attaching the antibody or antibody fragment to a therapeutic agent to form a complex, and administering the complex to a subject with prostate cancer. Identifying an endogenous homologue for the isolated peptide comprises obtaining an antibody to the peptide, and identifying the homologue with the antibody. The homologue is ***purified*** with the antibody. Treatment of a disease state comprises: (a) selecting a peptide targeted to cells associated with the disease state; (b) attaching to the peptide one or more molecules for treating the disease state; and (c) administering the peptide to a subject with the disease, where the disease is diabetes, an inflammatory disease, arthritis, atherosclerosis, cancer, autoimmune disease, bacterial infection, viral infection, cardiovascular disease or degenerative disease. Identifying targeting peptides against antibodies from an individual with a disease state comprises obtained a sample from the individual, obtaining antibodies from the sample, adding a phage display library to the antibodies, and collecting phage bound to the antibodies. The ***method*** further comprises coupling the antibodies to a solid support prior to adding the phage display library. The solid support comprises protein G attached to beads. The phage library is added to the antibodies obtained from an individual who does not have a disease state, and the phage binding to the antibodies from the library is removed, and the remaining phage is added to antibodies from an individual having a disease. Antibodies against one or more targeting peptides are obtained by preparing one or more targeting peptides, immobilizing the peptides on a solid support, exposing the peptides to a sample containing antibodies, and collecting antibodies that bind to the peptides. Blocking metastasis comprises screening a phage library against a sample of ascites from an individual with cancer, obtaining at least one targeting peptide that binds to an antibody from the sample, and administering the peptide to a patient with cancer. The peptide is administered into the peritoneal cavity of the patient. The cancer is ovarian cancer, primary peritoneal cancer, mesothelioma, Mullerian cancer, liver cancer or gastrointestinal cancer. Preferred Vector: The phage comprised in the gene therapy vector is fUS5 phage. The targeting peptide is expressed as part of the PIII or PVIII coat proteins. The gene therapy vector can be a chimeric phage comprising elements from adeno-associated virus, and further comprises a gene encoding a therapeutic protein or peptide. The vector comprising the

nucleic acid encoding the peptide is a prokaryotic or a eukaryotic expression vector, selected from a plasmid, a cosmid, a yeast artificial chromosome, a bacterial artificial chromosome, a virus, and a phage. Preferred Peptide: The isolated peptide is 5, 7, 10, 25 or 50 amino acids or less in size, and comprises at least 5 contiguous amino acids of a sequence selected from 255 amino acid sequences (not defined in the specification). The peptide is covalently attached to a molecule, which is selected from a drug, a chemotherapeutic agent, a radioisotope, a pro-apoptosis agent, an antiangiogenic agent, a hormone, a cytokine, a growth factor, a cytotoxic agent, a peptide, a protein, an antibiotic, an antibody, a Fab fragment of an antibody, an imaging agent, an antigen, a survival factor, an anti-apoptotic agent, and a hormone antagonist. The pro-apoptosis agent is selected from gramicidin, magainin, mellitin, defensin, cecropin, (KLAKLAK)2, (KLAKKLA)2, (KAAKKAA)2, and (KLGKKLG)3. The anti-angiogenic agent is thrombospondin, angiostatin5, pigment epithelium derived factor, angiotensin, laminin peptides, fibronectin peptides, ***plasminogen*** ***activator*** inhibitors, tissue metalloproteinase inhibitors, interferons, interleukin 12, platelet factor 4, IP-10, Gro-beta, thrombospondin, 2-methox yoestradiol, proliferin-related protein, carboxiamidotriazole, CM101, Marimastat, pentosan polysulphate, angiopoietin 2 (Regeneron), interferon-alpha, herbimycin A, PNU145156E, 16K prolactin fragment, Linomide, thalidomide, pentoxifylline, genistein, TNP-470, endostatin, paclitaxel, accutin, cidofovir, vincristine, bleomycin, AGM-1470, platelet factor 4 or minocycline. The cytokine is selected from interleukin (IL) 1, 1L-2, IL-5, IL-10, 1L-11, IL-12, 1L-18, interferon-gamma (IF-gamma), IF-alpha, IF-beta, tumor necrosis factor-alpha (TNF-alpha), or granulocyte macrophage colony stimulating factor (GM-CSF). The peptide is attached to a virus, a bacteriophage, a bacterium, a liposome, a microparticle, a magnetic bead, a microdevice, a yeast cell, a mammalian cell, a cell, a eukaryotic expression vector, preferably a gene therapy vector, or to a solid support. The peptide may be alternatively attached to a molecule, such as a macromolecular complex.

ACTIVITY - Cytostatic; immunosuppressive; anti-inflammatory; antiarthritic; antiatherosclerotic; antidiabetic; antibacterial; antiviral.

MECHANISM OF ACTION - Peptide therapy.

USE - The ***methods*** and composition are useful for identifying targeting peptides and one or more receptors for a targeting peptide. The targeting peptides are used for selective delivery of therapeutic agents, including gene therapy vectors and fusion proteins, to specific organs, tissues, or cell types in subject. The targeting peptide may also be used for treating diseases such as diabetes mellitus, inflammatory diseases, arthritis, atherosclerosis, cancer, autoimmune diseases, bacterial and viral infections.

ADMINISTRATION - Administration is through oral, nasal, buccal, rectal, vaginal, topical, orthotopic, intradermal, subcutaneous, intramuscular, intraperitoneal, intraarterial or intravenous routes.

EXAMPLE - Fresh human ribs removed during surgery for access to underlying tumors were section to expose the bone marrow surface. Bone samples were washed with ice-cold DMEM/0.5% BSA containing protease inhibitors (DMEM/BSA-PI). Rib surfaces were incubated with DMEM/BSA-PI at 4degreesC for 40 min, and samples were then lifted and transferred to a petri dish. A large drop of library ***diluted*** in DMEM/BSA-PI was then added to the dish containing the sample. Phage library remained in contact with the bone marrow surface for 1-2 hrs. Tissue was washed gently about 5-10 times with 1 ml DMEM/BSA-PI. K91 ***E*** ***coli*** were infected and phage recovery was performed. Bone sample was removed after infection and aliquots of the K91 culture were plated in serial ***dilutions*** . Cultures were grown overnight, and ***processed*** for successive rounds of panning. Targeting peptides that homes to human bone marrow were the following: (1) CGLRCPLVCPGGC; (2) CPVGCGGGCRPAC; (3) CEVLCGVDCSNRC; (4) WVSPVLG; (5) VLGPRAM; (6) LVGKWPY; (7) PSRRLGS; (8) APNTPVL; (9) CRLADKELC; (10) CRLSLPELC; (11) TSFRSL; (12) SLHRVAR; (13) PLLVRTV; and (14) GRGRMTS. (297 pages)

L7 ANSWER 10 OF 47 BIOTECHNO COPYRIGHT 2005 Elsevier Science B.V. on STN
DUPLICATE
ACCESSION NUMBER: 2002:35420060 BIOTECHNO
TITLE: Design, production, and characterization of an

engineered biotin ligase (Bira) and its application for affinity purification of staphylokinase produced from Bacillus subtilis via secretion

AUTHOR: Wu S.-C.; Yeung J.C.; Hwang P.M.; Wong S.-L.

CORPORATE SOURCE: S.-L. Wong, Department of Biological Sciences, Division of Cellular Biology, University of Calgary, 2500 University Drive, N.W., Calgary, Alta. T2N 1N4, Canada.
E-mail: slwong@ucalgary.ca

SOURCE: Protein Expression and Purification, (2002), 24/3 (357-365), 42 reference(s)
CODEN: PEXPEJ ISSN: 1046-5928

DOCUMENT TYPE: Journal; Article

COUNTRY: United States

LANGUAGE: English

SUMMARY LANGUAGE: English

AN 2002:35420060 BIOTECHNO

AB A major attraction in using Bacillus subtilis as an expression host for heterologous protein production is its ability to secrete extracellular proteins into the culture medium. To take full advantage of this system, an efficient ***method*** for recovering the target protein is crucial. For secretory proteins which cannot be ***purified*** by a simple scheme, in vitro biotinylation using biotin ligase (BirA) offers an effective alternative for their ***purification*** . The availability of large amounts of quality BirA can be critical for in vitro biotinylation. We report here the engineering and production of an Escherichia coli BirA and its application in the ***purification*** of staphylokinase, a fibrin-specific ***plasminogen*** ***activator*** , from the culture supernatant of Bacillus subtilis via in vitro biotinylation. BirA was tagged with both a chitin-binding domain and a hexahistidine tail to facilitate both its ***purification*** and its removal from the biotinylated sample. We show in this paper how, in a unique way, we solved the problem of protein aggregation in the ***E*** . ***coli*** BirA production system to achieve a yield of ***soluble*** functional BirA hitherto unreported in the literature. Application of this novel BirA to protein ***purification*** via in vitro biotinylation in general will also be discussed. Biotinylated staphylokinase produced in the study not only can act as an intermediate for easy ***purification*** , it can also serve as an important element in the creation of a blood clot targeting and dissolving agent. .COPYRGT. 2002 Elsevier Science (USA).

L7 ANSWER 11 OF 47 BIOTECHNO COPYRIGHT 2005 Elsevier Science B.V. on STN

ACCESSION NUMBER: 2002:36330119 BIOTECHNO

TITLE: Bacterial antigen-induced release of white cell- and platelet-derived bioactive substances in vitro

AUTHOR: Hammer J.H.; Mynster T.; Rosendahl S.; Reimert C.M.; Brunner N.; Skov F.; Nielsen H.J.

CORPORATE SOURCE: H.J. Nielsen, Dept. of Surg. Gastroenterology 435, Hvidovre University Hospital, DK-2650 Hvidovre, Denmark.
E-mail: h.j.nielsen@ofir.dk

SOURCE: International Journal of Gastrointestinal Cancer, (2002), 31/1-3 (165-179), 39 reference(s)
CODEN: IJGCAJ ISSN: 0169-4197

DOCUMENT TYPE: Journal; Article

COUNTRY: United States

LANGUAGE: English

SUMMARY LANGUAGE: English

AN 2002:36330119 BIOTECHNO

AB Objectives. Poor prognosis after resection of primary colorectal cancer may be related to the combination of perioperative blood transfusion and subsequent development of infectious complications. Various white cell- and platelet-derived cancer-growth substances may be involved in this ***process*** . Therefore, we studied the in vitro release of substances from white cells and platelets stimulated by bacterial antigens and supernatants from stored red-cell components. ***Methods*** . Eight units of whole blood (WB) and 8 U of buffy-coat-depleted red-cell (SAGM) blood were donated by healthy blood donors. Subsequently, one-half of each unit was leucocyte-depleted by filtration, and all 32 half-units

were stored under standard conditions for 35 d. Just after storage, and on d 7, 21, and 35 during storage, aliquots of the supernatants were removed from the units and frozen at -80.degree.C. WB from other healthy donors was stimulated for 2 h with sodium chloride (controls), with Escherichia coli (***E*** . ***coli***) lipopolysaccharide (LPS) alone, or with LPS plus supernatants from the WB units (***diluted*** 1:10), or from the SAGM units (***diluted*** 1:20) stored for 0, 7, 21, or 35 d, respectively. Similar assays were performed using Staphylococcus aureus-derived protein A as a stimulatory antigen. The concentration of eosinophil cationic protein (ECP), myeloperoxidase (MPO), histamine (HIS), and ***plasminogen*** - ***activator*** inhibitor-1 (PAI-1) were determined in supernatants from the stored blood and in assay supernatants by using enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA) ***methods*** . Results. The extracellular concentration of ECP, MPO, and HIS increased significantly in a storage-time-dependent manner in nonfiltered WB and SAGM blood, and the increase was abrogated by prestorage leukofiltration. Similarly, PAI-1 increased significantly in nonfiltered WB, and the increase was abrogated by prestorage leukofiltration. The supernatant concentrations of the four substances were significantly increased in LPS-stimulated (0.5-4 fold) and in protein A-stimulated (0.5-13.5-fold) assays compared with controls. The addition of supernatants from stored nonfiltered WB or SAGM blood significantly increased the assay supernatant of ECP, MPO, HIS, and PAI-1 concentrations storage-time-dependently in LPS-stimulated assays. Prestorage leukofiltration abrogated the additional effect of supernatants from stored blood. Similar results were observed for ECP and HIS through the addition of supernatants from stored blood to protein A-stimulated assays. Protein A stimulation did not lead to increased PAI-1 release in assays ***diluted*** by supernatants from stored blood. However, the MPO concentrations were significantly ($p = 0.004$), and independent of storage time and leukofiltration, increased in protein A-stimulated assays ***diluted*** by supernatants from stored blood compared with sodium chloride ***dilution*** . Conclusion. Extracellular ECP, MPO, HIS, and PAI-1 accumulate during Storage of nonfiltered red-cell components, but the accumulation can be prevented by prestorage leukofiltration. In addition, bacterial antigens appear to induce significant release of the substances from white cells and platelets. Addition of supernatants from stored, nonfiltered WB and SAGM blood may increase the substance levels in a storage-time-dependent manner, and prestorage leukofiltration may prevent further increase by supernatants, except for MPO.

L7 ANSWER 12 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2001-02080 BIOTECHDS
TITLE: Preparation of water-soluble eukaryotic proteins with disulfide bridges e.g. recombinant plasminogen-activator, comprises cultivation of prokaryotic cells in the presence of arginine or amide compound;
involving vector plasmid pET20 and plasmid pUBS520-mediated gene transfer for expression in Escherichia coli
PATENT ASSIGNEE: Roche
LOCATION: Basle, Switzerland.
PATENT INFO: EP 1048732 2 Nov 2000
APPLICATION INFO: EP 1999-107412 26 Apr 1999
PRIORITY INFO: EP 1999-107412 26 Apr 1999
DOCUMENT TYPE: Patent
LANGUAGE: German
OTHER SOURCE: WPI: 2000-674185 [66]
AN 2001-02080 BIOTECHDS
AB Preparation of water- ***soluble*** , naturally occurring eukaryotic proteins containing two or more cysteine units bound via a disulfide bridge, comprises cultivation of prokaryotic cells in the presence of arginine or an amide compound (claimed). The ***method*** is useful for the preparation of eukaryotic proteins e.g. proteases, interferons, protein hormones, antibodies or antibody fragments (e.g. single chain FV fragment that binds to thyroid stimulating hormone). It is especially useful for preparing proteins with more than five disulfide bridges, e.g. recombinant ***plasminogen*** - ***activator*** . The technique is simple and does not require in vitro after-treatment, such as the removal

of ***inclusion*** bodies, reduction or naturization. The signal sequence originates from a Gram-neg. bacterium such as Escherichia coli. The prokaryotic cell contains a second expression vector that codes for a molecule chaperone, preferably from ***E*** . ***coli*** or heat shock protein-25. The DNA coding for the chaperone molecule is coupled with a DNA fragment that codes for a signal peptide which penetrates the inner bacterial membrane. (40pp)

L7 ANSWER 13 OF 47 BIOTECHNO COPYRIGHT 2005 Elsevier Science B.V. on STN
DUPLICATE
ACCESSION NUMBER: 2000:30035436 BIOTECHNO
TITLE: Refolding and purification of a urokinase plasminogen activator fragment by chromatography
AUTHOR: Fahey E.M.; Chaudhuri J.B.; Binding P.
CORPORATE SOURCE: E.M. Fahey, Department Chemical Engineering, University of Bath, Bath BA2 7AY, United Kingdom. E-mail: cesjbc@bath.ac.uk
SOURCE: Journal of Chromatography B: Biomedical Sciences and Applications, (2000), 737/1-2 (225-235), 37 reference(s)
CODEN: JCBBEP ISSN: 0378-4347
PUBLISHER ITEM IDENT.: S0378434799003606
DOCUMENT TYPE: Journal; Conference Article
COUNTRY: Netherlands
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 2000:30035436 BIOTECHNO
AB A fragment of recombinant ***urokinase*** ***plasminogen*** ***activator*** (u-PA), was expressed in ***E*** . ***coli*** in the form of ***inclusion*** bodies. ***Purification*** and ***renaturation*** was achieved in a three-stage ***process*** . Capture of the ***inclusion*** bodies was achieved by coupling wash steps in Triton X-100 and ***urea*** with centrifugation. ***Solubilised*** ***inclusion***. bodies were then ***renatured*** by buffer exchange performed by size-exclusion chromatography (SEPROS). Use of size-exclusion media with higher fractionation ranges resulted in an increase in the recovery of u-PA activity, to a maximum fractionation range of M(r) 10 000-1 500 000 after which recovery is reduced, due to a low resolution between the ***refolded*** u-PA and ***denaturant*** . Fractions of ***refolded*** u-PA were concentrated using cation ion-exchange chromatography, which selectively binds correctly folded u-PA. The result is concentrated, active, homogeneous u-PA. Copyright (C) 2000 Elsevier Science B.V.

L7 ANSWER 14 OF 47 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 3
ACCESSION NUMBER: 1999:490378 BIOSIS
DOCUMENT NUMBER: PREV199900490378
TITLE: Construction, expression and purification of plasminogen activator inhibitor type - 2 mutant, PAI - 2 DELTA CD in E. coli.
AUTHOR(S): Tian Yu [Reprint author]; Song Houyan [Reprint author]; Zhu Yunsong [Reprint author]
CORPORATE SOURCE: Laboratory of Molecular Genetics, School of Basic Medical Sciences, Shanghai Medical University, Shanghai, 200032, China
SOURCE: Journal of Shanghai Medical University, (July, 1999) Vol. 26, No. 4, pp. 235-238. print.
CODEN: SYDXEE. ISSN: 0257-8131.
DOCUMENT TYPE: Article
LANGUAGE: Chinese
ENTRY DATE: Entered STN: 16 Nov 1999
Last Updated on STN: 16 Nov 1999
AB Purpose To construct and express human ***plasminogen*** ***activator*** inhibitor type - 2 (PAI - 2) mutant, PAI - 2 DELTA CD in ***E*** . ***coli*** and establish a ***method*** for the ***purification*** of the recombinant protein (rhPAI - 2 DELTA CD). Methods PCR was used to generate the mutant, PAI - 2 DELTA CD. After restriction enzyme analysis and DNA sequencing, the mutant gene was

ligated with prokaryotic expression vector pLY - 4 and transfected a special strain of E. coli. The expression product was identified by Western Blot and the PAI activity assay. After fermentation, the bacterial cells were homogenized at the pressure of 50 MPa. The PAI - 2 mutant was purified by a protocol including Q - Sepharose ion-exchange chromatography, hydrophobic interaction chromatography and Sephadex G - 75 gel filtration. Results The expressed product was about 15% of total bacteria proteins, and was identical to the mutant of PAI-2 with respect to interaction with PAI - 2 polyclonal antibody. The mutant could inhibit urokinase-type plasminogen activitor measured by milk-agarose plate assay and reverse milk-agarose zymograph. After purification, the purity of rhPAI - 2 DELTA CD was up to 97%, the protein yielding was 36 mg per liter of culture, the specific activity was 28 640 AIU/mg. Conclusions The construction and expression of PAI - 2 DELTA CD in E. coli was successful.

L7 ANSWER 15 OF 47 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1998:756962 CAPLUS
DOCUMENT NUMBER: 130:76813
TITLE: Expression and purification of human plasminogen activator inhibitor type-2 in Escherichia coli
AUTHOR(S): Tian, Yu; Shen, Jun-Qing; Li, Ping; Song, Hou-Yan; Zhu, Yun-Song
CORPORATE SOURCE: Lab. Mol. Genetics, Shanghai Med. Univ., Shanghai, 200032, Peop. Rep. China
SOURCE: Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao (1998), 14(5), 536-541
CODEN: ZSHXF2; ISSN: 1007-7626
PUBLISHER: Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao Bianweihui
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB To express human ***plasminogen*** ***activator*** inhibitor type-2 (PAI-2) in ***E*** . ***coli*** and establish a ***method*** for the ***purifn*** . of the recombinant protein (rhPAI-2), PAI-2 cDNA was amplified by PCR. After restriction enzyme anal. and DNA sequencing, the PCR product was ligated with prokaryotic expression vector. The sol. rhPAI-2 was purified by a protocol which included ammonium sulfate pptn., Sephadex G-75 gel filtration, Q-sepharose ion-exchange chromatog. and hydrophobic interaction chromatog. The expressed recombinant PAI-2 was about 14% of total bacteria protein, and was identical to PAI-2 with respect to interaction with polyclonal antibody. The purity of rhPAI-2 was up to 90%, the protein yielding 30 mg per L of culture, the specific activity of rhPAI-2 was 11,866 AIU/mg, and rhPAI-2 was found to inhibit u-PA by forming SDS-resistant complex.

L7 ANSWER 16 OF 47 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 4
ACCESSION NUMBER: 1998:386347 BIOSIS
DOCUMENT NUMBER: PREV199800386347
TITLE: Purification and biochemical characteristics of human plasminogen activator inhibitor type-2 expressed in E. coli.
AUTHOR(S): Tian, Yu; Shen, Junqing; Cai, Xiaoyan; Li, Ping; Song, Houyan; Zhu, Yunsong
CORPORATE SOURCE: Lab. Mol. Genet., Sch. Basic Med. Sci., Shanghai Med. Univ., Shanghai 200032, China
SOURCE: Acta Academiae Medicinae Shanghai, (June, 1998) Vol. 25, No. 3, pp. 163-166. print.
CODEN: SYDXEE. ISSN: 0257-8131.
DOCUMENT TYPE: Article
LANGUAGE: Chinese
ENTRY DATE: Entered STN: 10 Sep 1998
Last Updated on STN: 21 Oct 1998
AB PURPOSE. To establish a ***method*** for the ***purification*** of recombinant human ***plasminogen*** ***activator*** inhibitor type - 2 (rhPAI - 2) expressed in ***E*** . ***coli*** and to study several aspects of biochemical characteristics of rhPAI - 2. METHODS. Soluble rhPAI - 2 was about 14 % of the total bacteria protein and was purified by a protocol which includes ammonium sulfate precipitation, Sephadex G - 75 gel filtration, Q - sepharose ion-exchange chromatography

and hydrophobic interaction chromatography. rhPAI - 2 was renatured and its thermal - stability, pH - stability, guanidinium - stability, H2O2 - stability and the second - order rate constant were measured by chromogenic assay. The complex formation between rhPAI - 2 and u - PA was determined by SDS PAGE. RESULTS. The purity of rhPAI - 2 was up to 90%, protein yielding was 19.2% and specific activity, 11 866 AIU/mg. After refolding, rhPAI - 2 was found to inhibit u - PA by forming SDS - resistant complex and the second - order rate constant raging over (2.2 apprx 2.6) X 106 (mol/L)-1 cntdot s-1 for u-PA. When temperature was up, to 60degreeC and pH down to 3, rhPAI - 2 was totally inactivated. When the concentration of guanidinium and H2O2 was up to 1.5 mol/L and 0.5 mol/L respectively, the relative activity of rhPAI - 2 was still remain to 90%. CONCLUSIONS. The purification of rhPAI - 2 is efficient and successful and the biochemical characteristics of recombinant human PAI - 2 are -much like the natural PAI - 2.

L7 ANSWER 17 OF 47 BIOTECHNO COPYRIGHT 2005 Elsevier Science B.V. on STN
DUPLICATE
ACCESSION NUMBER: 1998:28183997 BIOTECHNO
TITLE: Production of a hybrid protein consisting of the N-terminal fragment of urokinase and the C-terminal domain of urinary trypsin inhibitor in Escherichia coli
AUTHOR: Sugino D.; Okushima M.; Kobayashi H.; Terao T.
CORPORATE SOURCE: D. Sugino, Nissin Central Research Institute, Nojicho 2247, Kusatsu, Shiga 525, Japan.
SOURCE: Biotechnology and Applied Biochemistry, (1998), 27/2 (145-152), 24 reference(s)
CODEN: BABIEC ISSN: 0885-4513
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1998:28183997 BIOTECHNO
AB We have constructed a hybrid protein (ATFHI) consisting of an N-terminal fragment from ***urokinase*** (ATF) and HI-8, which is the C-terminal domain of urinary trypsin inhibitor. The fusion genes for the hybrid proteins were engineered by PCR and cloned into expression plasmids. Under the control of the tac promoter, fusion genes were efficiently expressed in Escherichia coli. The hybrid proteins, produced as ***inclusion*** bodies in ***E*** . ***coli*** , were ***refolded*** by a dialysis ***method*** and ***purified*** by ion-exchange chromatography. ATFHI exhibited bifunctional activity related to antimetastatic effects: the ***urokinase*** receptor-binding activity of ATF and the inhibitory activity of HI- 8 on plasmin.

L7 ANSWER 18 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1997-06395 BIOTECHDS
TITLE: Bacterium producing eukaryotic proteins in soluble form; thioredoxin, glutaredoxin or protein-disulfide-isomerase thioredoxin-like domain gene cloning and co-expression with target protein in Escherichia coli
AUTHOR: Ishii S; Yura T
PATENT ASSIGNEE: HSP-Res.Inst.Osaka; Inst.Phys.Chem.Res.Saitama
LOCATION: Osaka, Japan; Saitama, Japan.
PATENT INFO: EP 768382 16 Apr 1997
APPLICATION INFO: EP 1996-116359 11 Oct 1996
PRIORITY INFO: JP 1995-291859 13 Oct 1995
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1997-214818 [20]
AN 1997-06395 BIOTECHDS
AB A new ***method*** for ***soluble*** recombinant protein production involves transformation of a bacterium (e.g. Escherichia coli BL21 (DE3)/Trx-Myb (FERM BP-5670)) with separate vectors or a single vector encoding ***E*** . ***coli*** or human thioredoxin (or glutaredoxin or protein-disulfide-isomerase (EC-5.3.4.1) thioredoxin-like domain) and interferon, interleukin, interleukin receptor, interleukin receptor-antagonist, colony stimulating factor, erythropoietin,

thrombopoietin, leukemia-inhibitory factor, stem cell factor, tumor necrosis factor, somatotropin, ***pro*** -insulin, insulin-like growth factor, fibroblast growth factor, platelet-derived growth factor, transforming growth factor, hepatocyte growth factor, bone morphogenetic protein, nerve growth factor, neurotrophic factor, neurotrophin-3, ***urokinase*** , tissue ***plasminogen*** - ***activator*** , blood-clotting factor, protein-C, glucosylceramidase, superoxide-dismutase, renin, lysozyme, cytochrome-P450, ***pro*** -chymosin, trypsin-inhibitor, elastase-inhibitor, lipocortin, Ig, single chain antibody, complement, serum albumin, virus protein, oncoprotein or transcription factor. (15pp)

L7 ANSWER 19 OF 47 BIOTECHNO COPYRIGHT 2005 Elsevier Science B.V. on STN
DUPLICATE
ACCESSION NUMBER: 1997:27298750 BIOTECHNO
TITLE: Laboratory-scale permeabilization of Escherichia coli cells for recovery of a small recombinant protein - Staphylokinase
AUTHOR: Gehmlich I.; Pohl H.D.; Knorre W.A.
CORPORATE SOURCE: I. Gehmlich, Hans-Knoll-INF, Bereich Bioverfahrensentwicklung, Beutenbergstr. 11, 07745 Jena, Germany.
SOURCE: Bioprocess Engineering, (1997), 17/1 (35-38), 13 reference(s)
CODEN: BIENEU ISSN: 0178-515X
DOCUMENT TYPE: Journal; Article
COUNTRY: Germany, Federal Republic of
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1997:27298750 BIOTECHNO
AB The recovery of recombinant proteins includes a ***purification*** ***process*** that has to be compressed to a minimum of steps in order to get high yields with a low cost expenditure. A selective liberation of recombinant proteins by cell permeabilization leads to both a high product purity just in the beginning of the recovery ***process*** and to a simplification of the cell residue separation compared to the mechanical cell disruption. In case of the ***purification*** of the bacterial ***plasminogen*** ***activator*** Staphylokinase from ***E*** . ***coli*** cells, yields of 82% with a purity of 46% were attained by utilization of permeabilization by biomass freezing, resuspension in a Tris/EDTA-buffer and following micro-diafiltration. A recovery ***process*** without interruption (freezing) is possible due to the addition of ***guanidine*** -HCl and Triton X100 to the buffer. These ***methods*** were developed on a laboratory-scale.

L7 ANSWER 20 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1996-15077 BIOTECHDS
TITLE: Hybridization analysis for the quantitative determination of residual DNA in some recombinant proteins expressed in E. coli cells;
quality control for e.g. human recombinant pro-urokinase, basic fibroblast growth factor or granulocyte-macrophage colony stimulating factor production from Escherichia coli
AUTHOR: Facchetti I; Schito S; Vigevani A
CORPORATE SOURCE: Pharmacia
LOCATION: R & D Analytical Chemistry, Pharmacia Pharmaceuticals Milano, Via Papa Giovanni XXIII n. 23, 20014 Nerviano, Italy.
SOURCE: Boll.Chim.Farm.; (1996) 135, 5, 287-96
CODEN: BCFAAI
ISSN: 0006-6648
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 1996-15077 BIOTECHDS
AB Quantitative DNA hybridization for biopharmaceutical quality control was tested on 3 recombinant proteins (human ***pro*** - ***urokinase*** , human basic fibroblast growth factor and granulocyte-macrophage colony stimulating factor, all expressed in Escherichia coli. Genomic and plasmid DNA were first isolated from ***E*** . ***coli*** cells, followed by construction of a ***dilution*** series of genomic and plasmid DNA to set up a standard curve, heat ***denaturation*** and

dot-blotting, linearization of plasmid and genomic DNA to generate probes, DNA probe radiolabel with alpha-32P-dCTP, heat ***denaturation*** , and dot-blot hybridization with the samples. The test could be used to measure DNA at trace levels, and was validated for biotechnological proteins with different features. Detection limits (0.06 pg for plasmid DNA and 5 pg for genome DNA) complied with FDA requirements. Interference due to sample protein, salts and phenol were avoided by using an improved ***purification*** ***method*** . High DNA probe specificity resulted from very low intensity signals derived from interaction with heterologous nucleic acids. (30 ref)

L7 ANSWER 21 OF 47 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 7
ACCESSION NUMBER: 1996:30999 SCISEARCH
THE GENUINE ARTICLE: TM264
TITLE: Purification and characterization of recombinant pro-urokinase
AUTHOR: Ma Z (Reprint); Yu R R; Chen X C; Hua Z C; Zhu D X
CORPORATE SOURCE: NANJING UNIV, NATL KEY LAB PHARMACEUT BIOTECHNOL, DEPT BIOCHEM, NANJING 210093, PEOPLES R CHINA
COUNTRY OF AUTHOR: PEOPLES R CHINA
SOURCE: ACTA BIOCHIMICA ET BIOPHYSICA SINICA, (NOV 1995) Vol. 27, No. 6, pp. 670-674.
ISSN: 0582-9879.
PUBLISHER: SHANGHAI INST BIOCHEMISTRY, ACADEMIA SINICA, 320 YUE-YANG ROAD, SHANGHAI 20031, PEOPLES R CHINA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: Japanese
REFERENCE COUNT: 6
ENTRY DATE: Entered STN: 1996
Last Updated on STN: 1996
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB A ***method*** to ***purify*** recombinant ***pro*** - ***urokinase*** from ***renaturated*** product expressed in ***E*** . ***coli*** was reported. Through selected precipitation by Zn2+, affinity chromatography and affinity adsorption by benzamidine, the recombinant product was purified and its specific activity mss 110000 IU/mg, Results from SDS-PAGE: showed that the purified protein, ender reduced or nonreduced conditions migrated as a single band with a molecular weight of 43 kd. Kinetic studies showed that its K-m, was 45 mu M. In vitro fibrin clot lysis verified that the recombinant pro-UK had better fibrin selectivity than the natural H-UK.

L7 ANSWER 22 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1996-02631 BIOTECHDS
TITLE: Improved method for pro-urokinase refolding with inclusion body from recombinant Escherichia coli; protein renaturation
AUTHOR: Kubo M; Nishi A
CORPORATE SOURCE: Numazu-Coll.Technol.; Tosoh
LOCATION: Department of Chemistry and Biochemistry, Numazu College of Technology, 2700 Ooka, Numazu-shi, Shizuoka-ken 410, Japan.
SOURCE: J.Ferment.Bioeng.; (1995) 80, 6, 622-24
CODEN: JFBIEX
ISSN: 0922-338X
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 1996-02631 BIOTECHDS
AB An efficient ***method*** for ***pro*** - ***urokinase*** (EC-3.4.21.73) protein ***renaturation*** from recombinant Escherichia coli K12 JM103 (plasmid pMUT4L) ***inclusion*** bodies was developed. Expression was carried out with 0.1 mM IPTG. The protein was efficiently ***refolded*** when heat treatment was applied to a protein ***denaturing*** solution (50 mM Tris-HCl, pH 8.0, containing 8 M ***guanidine*** -HCl). The total enzyme activity and specific activity in response to the 50 deg heat treatment compared to normal ***methods*** (25 deg) were enhanced by 10% and 25%, respectively. Enhanced protein ***refolding*** was also observed in the case of a reduced protein concentration in the protein ***refolding*** solution. Results indicate that correct protein folding is closely

related to the protein concentration in the ***refolding*** solution. This ***method*** may be applicable to other ***inclusion*** body-forming proteins from recombinant ***E*** . ***coli*** . (17 ref)

L7 ANSWER 23 OF 47 LIFESCI COPYRIGHT 2005 CSA on STN
ACCESSION NUMBER: 96:34365 LIFESCI
TITLE: Active site labeling with dansyl-glutamyl-glycyl-arginyl chloromethyl ketone demonstrates the full activity of the refolded and purified tissue-type plasminogen activator variant BM 06.022
AUTHOR: Kohnert, U.; Wozny, M.; Llinas, M.; Roos, A.; Fischer, S.
CORPORATE SOURCE: Boehringer Mannheim GmbH, Biochem. Res. Cent. Penzberg, Nonnenwald 2, D-82377 Penzberg, Germany
SOURCE: APPL. BIOCHEM. BIOTECHNOL., (1995) vol. 55, no. 2, pp. 157-166.
ISSN: 0273-2289.
DOCUMENT TYPE: Journal
FILE SEGMENT: W3
LANGUAGE: English
SUMMARY LANGUAGE: English
AB BM 06.022 is a tissue-type ***plasminogen*** ***activator*** deletion variant that is comprised of the kringle 2 and the protease domain of the native molecule. BM 06.022 is expressed as inactive ***inclusion*** bodies in ***E*** . ***coli*** and transferred into the active enzyme by an in vitro folding ***process*** . Active site labeling with dansyl-glutamyl-glycyl-arginyl chloromethyl ketone provides evidence that the ***purified*** BM 06.022 is fully active and that misfolded species are completely removed by affinity chromatography on ETI-Sepharose. The comparison of the kinetics of the inhibition of BM 06.022 with that of CHO-t-PA indicates that the active centers of both enzymes are rather similar. The further evaluation of the site of interaction of BM 06.022 and DnsEGRck by mass spectroscopy and amino acid sequence analysis revealed that the inhibitor is bound selectively to His sub(322), which is part of the catalytic triad of this serine protease.

L7 ANSWER 24 OF 47 LIFESCI COPYRIGHT 2005 CSA on STN
ACCESSION NUMBER: 96:48888 LIFESCI
TITLE: Functional display of proteins, mutants proteins, fragments of proteins and peptides on the surface of filamentous (bacterio) phages: A review
AUTHOR: Pannekoek, H.; Van Meijer, M.; Gaardsvoll, H.; Jan van Zonneveld, A.
CORPORATE SOURCE: Univ. Amsterdam, Acad. Med. Cent., Dep. Biochem., Amsterdam, The Netherlands
SOURCE: CYTOTECHNOLOGY, (1995) vol. 18, no. 1-2, pp. 107-112.
ISSN: 0920-9069.
DOCUMENT TYPE: Journal
TREATMENT CODE: General Review
FILE SEGMENT: W3
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Cytoplasmic expression of complex eukaryotic proteins in Escherichia coli usually yields inactive protein preparations. In some cases, (part) of the biological activity can be recovered by rather inefficient ***denaturation*** - ***renaturation*** procedures. Recently, novel concepts have been developed for the expression of fully functional eukaryotic proteins in ***E*** . ***coli*** . Essential to the success of these procedures is the transport of such proteins across the inner membrane to the periplasmic space, allowing proper folding and the establishment of disulfide bonding. Subsequently, fully functional proteins can be exposed on the surface of filamentous (bacterio)phages, provided a system is employed that consists of a cloning vector (e.g. the phagemid pComb3, Barbas et al., 1991) that generates phage particles in the presence of a helper phage. The main advantage of surface display of recombinant proteins is to facilitate the screening of very large numbers of different molecules by simple selection ***methods*** ("panning"). In addition, periplasmic expression yields relatively large quantities (e.g. 1 mg l super(-1) of culture) ***soluble*** protein. In this

review, the principle aspects of this novel expression system based on the phagemid pComb3 will be discussed. Two examples for functional periplasmic expression of human proteins in ***E*** . ***coli*** will be presented, namely i) the antigen binding moiety (Fab fragment) of human immunoglobulin (IgGs) and ii) the human ***plasminogen*** ***activator*** inhibitor 1, an essential regulator of the ***plasminogen*** activation system. Finally, perspectives for the application of this system to express mutant proteins, fragments of proteins and peptides are indicated.

L7 ANSWER 25 OF 47 BIOTECHNO COPYRIGHT 2005 Elsevier Science B.V. on STN
DUPLICATE
ACCESSION NUMBER: 1994:24315660 BIOTECHNO
TITLE: Interaction of high-molecular-weight basic fibroblast growth factor with endothelium: Biological-activity and intracellular fate of human recombinant M(r) 24,000 bFGF
AUTHOR: Gualandris A.; Urbinati C.; Rusnati M.; Ziche M.; Presta M.
CORPORATE SOURCE: Unit General Pathology and Imm., Dept. Biomedical Sc./Biotechnology, School Medicine, University Brescia,Brescia, Italy.
SOURCE: Journal of Cellular Physiology, (1994), 161/1 (149-159)
CODEN: JCLLAX ISSN: 0021-9541
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1994:24315660 BIOTECHNO
AB The single-copy gene of human basic fibroblast growth factor (bFGF) encodes four co-expressed isoforms, with an apparent molecular weight (M(r)) of 24 kD, 22.5 kD, 22 kD, and 18 kD, co-translated from a single mRNA. As a tool for the study of the role exerted by the different bFGF isoforms in the biology of endothelial cells, human recombinant 24-kD bFGF was produced and ***purified*** from transformed Escherichia coli cells. To this purpose, the novel CUG start codon present in human bFGF cDNA and responsible for the synthesis of 24-kD bFGF was mutagenized to the classic AUG start codon. Transient expression of the mutagenized cDNA in simian COS-1 cells, followed by immunolocalization and subcellular fractionation, resulted in the synthesis of high levels of 24-kD bFGF, which localizes in the cell nucleus as an intact protein. When the same 24-kD bFCF cDNA was expressed in ***E*** . ***coli*** , the recombinant protein was ***purified*** to homogeneity by heparin-Sepharose and ion-exchange chromatography. Recombinant 24-kD bFGF was similar to recombinant 18-kD bFGF in receptor-binding activity and in inducing cell proliferation, ***plasminogen*** ***activator*** production, and chemotactic movement in cultured endothelial cells. In agreement with the in vitro observations, 24-kD bFGF and 18-kD bFGF exerted a similar angiogenic response when assayed in vivo in the rabbit cornea. Experiments performed with the radiolabeled molecule demonstrated that 24-kD bFCF has an intrinsic ability to bind to high-affinity receptors when added to endothelial GM 7373 cell cultures. Receptor-bound 24-kD bFGF is internalized within the cell and associates with the nucleus with kinetics similar to 18-kD bFGF. Internalized 24-kD bFGF is first ***processed*** to the 18-kD form via a chloroquine-insensitive pathway and then to smaller fragments into the lysosomal compartment. At variance with the data obtained in transfected COS-1 cells, only limited amounts of exogenous internalized 24-kD bFGF associates with the nucleus in the intact form, mostly of the nuclear-bound molecule being represented by the ***processed*** 18-kD protein and by smaller degradation products. In conclusion, human recombinant 24-kD bFCF exerts a biological response in endothelial cells similar to 18-kD bFCF both in vitro and in vivo. Our data point to a different intracellular behavior of the high-molecular-weight bFCF isoform when added exogenously to cultured cells or when produced endogenously in transfected cells.

L7 ANSWER 26 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1993-04972 BIOTECHDS
TITLE: Process economics of animal cell and bacterial fermentations:

a case study analysis of tissue plasminogen-activator;
production in CHO cell culture and Escherichia coli
AUTHOR: Datar R V; Cartwright T; Rosen C G
CORPORATE SOURCE: Pall; TCS-Biol.; Abitec
LOCATION: Pall Corporation, 30 Sea Cliff Ave., Glen Cove, NY 11542, USA.
SOURCE: Bio/Technology; (1993) 11, 3, 349-57
CODEN: BTCHDA
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 1993-04972 BIOTECHDS
AB Production of recombinant tissue ***plasminogen*** - ***activator*** (tPA) was compared in CHO cells and in Escherichia coli K12. DHFR- CHO cells were transfected with plasmid pXL261 and grown in the presence of increasing quantities of methotrexate (MTX) for 5-7 days in a batch fermentation. Large segments of DNA adjacent to the DHFR gene were amplified, resulting in over-production of proteins whose genes were located in the amplified region. MTX-resistant clones were selected for ability to produce tPA. The CHO cells produced 33.5 mg tPA/l (47% yield). Human tPA cDNA was used in an ***E*** . ***coli*** expression vector based on the phage lambda left lytic promoter, plasmid pXL130. When tPA expression was repressed by warming to 42 deg, tPA was produced in an ***insoluble*** ***denatured*** form as 5-10% of the total cell protein. ***E*** . ***coli*** was grown in a batch fermentation for 1-2 days at 42 deg and produced 460 mg/l (2.8% yield) tPA. At ***refolding*** concentrations above 4 mg tPA/l, the best ***refolding*** yields were achieved at 20%. At concentrations between 2-3 mg tPA/l, a yield of 90% could be obtained based in the downstream ***processing*** strategy. (27 ref)

L7 ANSWER 27 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1993-00157 BIOTECHDS
TITLE: Separation of active and inactive/latent forms of PAI-1;
recombinant active plasminogen-activator-inhibitor-1 purification from Escherichia coli involves the separation of active and inactive/latent forms using cation-exchanger
PATENT ASSIGNEE: Du-Pont-Merck
PATENT INFO: WO 9216625 1 Oct 1992
APPLICATION INFO: WO 1992-US2066 19 Mar 1992
PRIORITY INFO: US 1991-671433 20 Mar 1991
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1992-349210 [42]
AN 1993-00157 BIOTECHDS
AB A ***method*** for separating active and inactive/latent forms of ***plasminogen*** - ***activator*** -inhibitor-1 (PAI-1) with a specific activity of more than 500,000 and less than 5,000 U/mg, respectively, in an S-2251 assay comprises: i. loading a sample containing a mixture of active and inactive/latent forms of PAI-1 to a cation-exchanger of 10-50 um particle size; and ii. eluting active and inactive/latent forms into separate fractions using a mobile phase buffer with a gradient of increasing ionic strength or increasing pH, or both, under conditions where active and inactive/latent forms of PAI-1 are eluted from the resin in separate fractions. Also claimed is a ***method*** using a mobile phase gradient of increasing ionic strength or decreasing pH, or both. In a ***method*** of extracting ***soluble*** recombinant PAI-1 expressed in Escherichia coli from lyzed host cells in a buffered solution, the improvement comprises using a buffered solution of 8-25 millisiemens ionic strength. The improvement is also claimed for extracting ***soluble*** recombinant PAI-1 expressed in ***E*** . ***coli*** host cells lyzed by sonication in a buffered solution. This improvement gives optimum extraction and yield. (25pp)

L7 ANSWER 28 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1992-07976 BIOTECHDS
TITLE: Purification of prourokinase;
from transformed Escherichia coli involves contaminant adsorption by gel chromatography
PATENT ASSIGNEE: Toyo-Soda

PATENT INFO: JP 04071488 6 Mar 1992
APPLICATION INFO: JP 1990-184197 13 Jul 1990
PRIORITY INFO: JP 1990-184197 13 Jul 1990
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 1992-128228 [16]
AN 1992-07976 BIOTECHDS
AB ***Purification*** of recombinant ***pro*** - ***urokinase*** (I) from Escherichia coli comprises contacting a sample with a polymer gel carrying an alkyl group at 20-50 deg, and contacting the gel with a solution prepared at below 10 deg to obtain (I) in the solution. This ***method*** enables the selective removal of contaminants and highly ***purified*** (I) is obtained. The column can be used repeatedly after washing alternatively with water and NaOH. In an example, (I) was ***purified*** from transformed ***E*** . ***coli*** KY1436 and dissolved in phosphate buffer (pH 7.0) containing 7% (NH4)2SO4 and 0.5 M ***guanidine*** -HCl. The protein concentration, ***urokinase*** (EC-3.4.21.31) activity and specific activity were 2.8 mg/ml, 92,00 U/ml and 32,860 U/mg, respectively. The sample solution was added to a gel (carrying a butyl group)-packed column. Buffer (200 ml) was added to elute contaminants. The same buffer was then added at RT and 4 deg. The flow rate was 2 ml/min. The protein concentration (mg/ml), at RT (and at 4 deg) were 0.8 (0.316, 200 (27,000), 250 (85,000), respectively. (5pp)

L7 ANSWER 29 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1992-07975 BIOTECHDS
TITLE: Purification of prourokinase; from transformed Escherichia coli involves contaminant adsorption by gel chromatography
PATENT ASSIGNEE: Toyo-Soda
PATENT INFO: JP 04071487 6 Mar 1992
APPLICATION INFO: JP 1990-184196 13 Jul 1990
PRIORITY INFO: JP 1990-184196 13 Jul 1990
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 1992-128227 [16]
AN 1992-07975 BIOTECHDS
AB ***Purification*** of recombinant ***prourokinase*** (I) from Escherichia coli comprises contacting a sample with a polymer gel carrying an alkyl group and contacting the gel with an alcohol series organic solvent to obtain (I) in the organic solvent. This ***method*** enables the selective removal of contaminants and highly ***purified*** (I) is obtained. The column can be used repeatedly after washing alternatively with water and NaOH. In an example, (I) was ***purified*** from transformed ***E*** . ***coli*** KY1436 and dissolved in phosphate buffer (pH 7.0) containing 7% (NH4)2SO4 and 0.5 M ***guanidine*** -HCl. The protein concentration, ***urokinase*** (EC-3.4.21.31) activity and specific activity were 2.8 mg/ml, 92,00 U/ml and 32,857 U/mg, respectively. The sample solution was added to a gel (carrying a butyl group)-packed column. After addition of 200 ml buffer to elute contaminants, 50 mM Tris-HCl buffer containing 0-10% ethanol was added. (I) fractions were eluted with 6% ethanol. The protein concentration, ***urokinase*** activity and specific activity were 0.2 mg/ml, 178,000 U/ml and 89,000 U/ml, respectively. The operation was carried out at a flow rate of 2 ml/min. (5pp)

L7 ANSWER 30 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1992-07696 BIOTECHDS
TITLE: Recovery of urokinase-like enzyme precursor; from Escherichia coli transformant by cell disintegration in the presence of a metal chelator without the need for cell separation
PATENT ASSIGNEE: Toyo-Soda
PATENT INFO: JP 04051893 20 Feb 1992
APPLICATION INFO: JP 1990-158568 19 Jun 1990
PRIORITY INFO: JP 1990-158568 19 Jun 1990
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 1992-109998 [14]
AN 1992-07696 BIOTECHDS

AB After culturing Escherichia coli, transformed with a plasmid containing a ***urokinase*** (EC-3.4.21.31)-like enzyme (I) gene, cells are crushed in the presence of a metal chelator without separation of the cells from the broth. The ***insoluble*** fraction is recovered and a protein ***renaturation*** ***process*** is carried out. Using this ***method*** , (I) can be recovered without separating cells from the broth. In an example, transformed ***E*** . ***coli*** KY1436 was grown in M9 medium containing glycerol and a casein degradation product to an OD-600 nm of 50. Tris-hydroxyaminomethane (12.1 g) and HCl were added to 1 l fermentation broth (50 g wet wt. cells) to adjust the pH to 9, after which ETDA (18.9 g) was added. After centrifugation (8,000 rpm, 20 min), the ***insoluble*** fraction was suspended in 0.1 M Tris-HCl buffer (pH 8, 500 ml). After addition of 8 M HCl- ***guanidine*** (500 ml), the mixture was incubated at 40 deg for 2 hr for ***solubilization*** . 50 mM Tris-HCl buffer (pH 8, 6 l) containing 5 mM EDTA, 0.2 M reduced-type glutathione and 0.02 mM oxidative-type glutathione was added for protein ***renaturation*** . Plasmin (EC-3.4.21.7) treatment yielded 271,000 U (I)/g wet wt. cells. (4pp)

L7 ANSWER 31 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1993-00121 BIOTECHDS
TITLE: Secretion of the t-PA protease domain in E. coli; human tissue plasminogen-activator gene cloning, expression in lon protease-deficient Escherichia coli and protein secretion using an OmpA signal peptide (conference abstract)
AUTHOR: Honeck T; Zacharias U; Hahn V; Will H
LOCATION: Max Delbrueck Centrum fuer Molekulare Medizin, Robert-Roessle-Str. 10, O-1115 Berlin, Germany.
SOURCE: Biol.Chem.Hoppe Seyler; (1992) 373, 9, 861-62
CODEN: BCHSEI
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 1993-00121 BIOTECHDS
AB 3 Different secretion vectors were tested for their ability to direct synthesis and export of the human tissue ***plasminogen*** - ***activator*** protease domain (tPA-protease) in Escherichia coli. A fusion between the protease cDNA and the ***E*** . ***coli*** outer membrane protein OmpA gene signal peptide sequence resulted in partial secretion of the gene product into the periplasm, as correctly ***processed*** and enzymatically active tPA-protease. Screening revealed that the lon protease-deficient strain ***E*** . ***coli*** CAG 597 at low temp. (25 deg) gave the best active enzyme yields. Selective ***purification*** of the ***processed*** , active protease was achieved by cell fractionation, followed by ionexchange and inhibitor affinity chromatography steps. The ***purified*** enzyme was homogeneous, as confirmed by SDS-PAGE, adsorption spectroscopy and active site titration. The kinetic properties were determined, and were compared with those of native t-PA. (2 ref)

L7 ANSWER 32 OF 47 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 9
ACCESSION NUMBER: 92252495 EMBASE
DOCUMENT NUMBER: 1992252495
TITLE: Distribution and pharmacokinetics of active recombinant plasminogen activator inhibitor-1 in the rat and rabbit.
AUTHOR: Racanelli A.L.; Diemer M.J.; Dobies A.C.; Mohamed S.N.; Reilly T.M.
CORPORATE SOURCE: Du Pont Merck Pharmaceut. Company, Cardiovascular Science, Experimental Station Building, Wilmington, DE 19880-0400, United States
SOURCE: Fibrinolysis, (1992) Vol. 6, No. 3, pp. 187-191.
ISSN: 0268-9499 CODEN: FBRIE7
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 025 Hematology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 920913

Last Updated on STN: 920913
AB Recombinant ***plasminogen*** ***activator*** inhibitor-1 (rPAI-1) produced and ***purified*** in active form from ***E*** . ***coli*** , was labelled with ([125])-iodine using the Bolton-Hunter ***method*** . The distribution of the radiolabelled protein was determined in the rat. The greatest percentage of ([125])I-rPAI-1/(g of tissue) was found in the liver, followed by the spleen, kidney, lungs and heart. Radioactivity was detected in the urine up to 9 h after injection of ([125])-rPAI-1. In addition, a crossover study was performed in rabbits to determine the pharmacokinetics of unlabelled rPAI-1 (100 .mu.g/kg i.v. and 2.5 mg/kg i.v.). PAI-1 antigen levels determined by an ELISA were used to calculate pharmacokinetic parameters using a two compartment open model. Significant dose dependent changes for t(1/2).alpha., V(d) and Cl(p) were observed while rPAI-1 cleared from the circulation in a biphasic manner with a t(1/2).beta. of approximately 15 min for both doses. Clearance of rPAI-1 activity closely paralleled the disappearance of the antigen. The pharmacokinetic profile of rPAI-1 with its short half-life, liver metabolism and limited tissue distribution may allow for the safe therapeutic use of rPAI-1 as an antifibrinolytic agent.

L7 ANSWER 33 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1991-08644 BIOTECHDS
TITLE: Pro-urokinase protein renaturation; recombinant protein expression in e.g. Escherichia coli
PATENT ASSIGNEE: Toyo-Soda
PATENT INFO: JP 03080078 4 Apr 1991
APPLICATION INFO: JP 1989-212981 21 Aug 1989
PRIORITY INFO: JP 1989-212981 21 Aug 1989
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 1991-144178 [20]
AN 1991-08644 BIOTECHDS
AB A ***method*** for ***renaturation*** of a ***urokinase*** precursor-like protein ((I) (***pro*** - ***urokinase*** , EC-3.4.21.31), which is produced as an ***insoluble*** recombinant protein in a host cell, involves contacting the protein with an aq. alkali solution and a sulfhydryl compound in the presence of a protein modifier. The pH of the solution is then lowered. The aq. alkali solution is an aq. solution of at least one organic base selected from monoethanolamine, diethylene tetramine, triethylene tetramine and tetraethylene pentamine, and the protein modifier is preferably ***urea*** (not more than 2 M). (I) is ***renatured*** in high efficiency by this ***method*** . In an example, Escherichia coli was transformed with a plasmid for the production of (I). ***E*** . ***coli*** containing (I) was crushed with a homogenizer and separated into ***insoluble*** and ***soluble*** fractions. 40 g Of the ***insoluble*** fraction was mixed with 40 ml of 8 M ***urea*** . The mixture was allowed to stand for 30 min, after which 80 ml of 10% triethylene tetramine was added to a pH of 11.5. After 30 min, reductive glutamine was added to 1 mM. The pH was adjusted to 9 with HCl for protein ***renaturation*** . (4pp)

L7 ANSWER 34 OF 47 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1991:156602 CAPLUS
DOCUMENT NUMBER: 114:156602
TITLE: Efficient renaturation and fibrinolytic properties of prourokinase and a deletion mutant expressed in Escherichia coli as inclusion bodies
AUTHOR(S): Orsini, Gaetano; Brandazza, Anna; Sarmientos, Paolo; Molinari, Antonio; Lansen, Jacqueline; Cauet, Gilles
CORPORATE SOURCE: Dep. Biotechnol., Farmitalia C, Erba, Milan, I-20146, Italy
SOURCE: European Journal of Biochemistry (1991), 195(3), 691-7
CODEN: EJBCAI; ISSN: 0014-2956
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Prourokinase is a plasminogen activator of 411 amino acids which displays clot-lysis activity through a fibrin-dependent mechanism, and which seems to be a promising agent for the treatment of acute myocardial infarction. The prepn. of recombinant prourokinase in bacteria has been hampered by

its insoly. and by difficulty in refolding the polypeptide chain. In this paper the authors describe the ***renaturation*** ***process*** of 2 recombinant proteins expressed in ***E*** . ***coli*** as ***inclusion*** bodies: ***prourokinase*** and a deletion deriv. (.DELTA.125- ***prourokinase***) in which 125 amino acids of the N-terminal region have been removed. Deletion of this sequence brings higher refolding yields and faster kinetics (first-order rate const. of renaturation of 0.57/h for .DELTA.125-prourokinase and 0.25/h for prourokinase). The process involves sequential steps of denaturation, redn. and controlled refolding of the polypeptide chain. When applied to pure, non-glycosylated and active prourokinase, it gives a refolding yield of about 80%, demonstrating the efficiency of the renaturation procedure. Lower yields (15 and 30%, resp., for prourokinase and .DELTA.125-prourokinase) were obtained when the same refolding protocol was applied to inclusion bodies from bacteria. After purifn. to homogeneity (as shown by HPLC and SDS/PAGE) specific activities were 160000 and 250000 IU/mg protein, resp., for prourokinase and .DELTA.125-prourokinase. As with prourokinase, the deletion mutant .DELTA.125-prourokinase displays a zymogenic nature, being activated by plasmin to the active two-chain form; however, this mutant is approx. 4-fold more resistant than prourokinase to plasmin activation, and consequently shows a different fibrinolytic profile.

L7 ANSWER 35 OF 47 BIOTECHNO COPYRIGHT 2005 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1991:21224084 BIOTECHNO
TITLE: Synthesis, purification and biological properties of a truncated mutant form of human tissue plasminogen activator produced in E. coli
AUTHOR: Fromage N.; Denefle P.; Cambou B.; Duchesne M.; Joyeux C.; Kovarik S.; Marin J.; Imbault F.; Uzan A.; Cartwright T.
CORPORATE SOURCE: Laboratoire de Biochimie des Macromolecules, Centre de Recherches de Vitry, Rhone Poulenc Sante, B.P. 14, 94403 Vitry Sur Seine, France.
SOURCE: Fibrinolysis, (1991), 5/3 (187-190)
CODEN: FBRIE7 ISSN: 0268-9499
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1991:21224084 BIOTECHNO
AB A truncated form of tissue ***plasminogen*** ***activator*** (t-PA) that lacks the two N-terminal domains of wild type t-PA was efficiently ***renatured*** and ***purified*** after expression in ***E*** . ***coli*** . The mutant t-PA was more ***soluble*** than wild type t-PA expressed as a nonglycosylated form in ***E*** . ***coli*** as was predicted by the elimination of a significant segment of predominantly hydrophobic sequence in the mutant. The efficiency of the ***renaturation*** ***process*** was, however, unchanged. In vitro biological activity of wild type and mutant t-PA was similar in chromogenic, fibrin plate and clot lysis assays. Both proteins also produced specific ***plasminogen*** activation in rabbits, however clearance of the mutant from the circulation was about four times slower than that of wild type t-PA. This confirms previous observations that the N-terminal domains contribute to the rapid clearance of t-PA, but are not essential for specific ***plasminogen*** activation in vivo.

L7 ANSWER 36 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1992-06193 BIOTECHDS
TITLE: Choosing a production system for recombinant proteins; human therapeutic produced by Escherichia coli, Saccharomyces cerevisiae, CHO cell culture (conference paper)
AUTHOR: Vapnek D
CORPORATE SOURCE: Amgen
LOCATION: Amgen Inc., Amgen Center, Thousand Oaks, CA 91320, USA.
SOURCE: Biol.Recombinant Microorg.Anim.Cells; (1991) Oholo 34 Meet., 1-14
DOCUMENT TYPE: Journal
LANGUAGE: English

AN 1992-06193 BIOTECHDS
AB Three major systems have been used to produce therapeutic proteins suitable for use in humans; Escherichia coli K12, Saccharomyces cerevisiae, and CHO cells, and the advantages and disadvantages of these systems were reviewed. Therapeutic proteins produced in ***E*** . ***coli*** include human somatotropin, insulin, interferon-alpha and-gamma, interleukin-1, -2 and -3, etc. Hepatitis B vaccine, insulin, epidermal growth factor and superoxide-dismutase (EC-1.15.1.1) have been produced in S. cerevisiae, and tissue ***plasminogen*** - ***activator*** , erythropoietin, Factor-VIII, etc. have been produced in CHO cells. Future developments in ***E*** . ***coli*** and S. cerevisiae systems will focus on the disadvantages such as the high level of endotoxin, protein expression in ***insoluble*** ***inclusion*** bodies, absence of N-terminal methionine ***processing*** and post-translation modification of proteins, with emphasis on developing new protein secretion systems. Future developments in CHO cells (and mammal cell vectors in general) will focus on the development of defined media, high cell density fermentations and versatile expression systems. (24 ref)

L7 ANSWER 37 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1991-00848 BIOTECHDS
TITLE: Activation of insoluble recombinant protein produced in transformed bacterium;
recombinant pro-urokinase activation after production in Escherichia coli by reducing agent e.g. 2-mercaptoethanol or dithiothreitol
PATENT ASSIGNEE: Toyo-Soda
PATENT INFO: JP 02227076 10 Sep 1990
APPLICATION INFO: JP 1989-273825 23 Oct 1989
PRIORITY INFO: JP 1989-273825 23 Oct 1989; JP 1988-267107 25 Oct 1988
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 1990-317324 [42]
AN 1991-00848 BIOTECHDS
AB During the ***processes*** of triturating ***insoluble*** recombinant proteins prepared in transformed bacteria, recovering the ***insoluble*** protein fraction from the triturated bacteria, and suspending and activating the fraction for isolation of the recombinant protein, a reducing agent in solution is added. The reducing agent, which is preferably 2-mercaptoethanol or dithiothreitol, is removed from the protein fraction before the activation step. The ***insoluble*** recombinant protein, preferably a ***prourokinase*** (EC-3.4.21.31) produced by transformed Escherichia coli, may be activated by the new ***method*** . In an example, transformed ***E*** . ***coli*** KY-1436 was incubated to produce human ***pro*** - ***urokinase*** . The bacterium was triturated and centrifuged, and the ***insoluble*** protein fraction was isolated, resuspended and centrifuged. 2-Mercaptoethanol (0.5 or 1.0%) was added to the enzyme isolate. The activity of the protein was increased to 678,000 U/mg wet wt. bacteria compared to 580,000 U/mg wet wt. bacteria for 1.0% and 0.5% mercaptoethanol treated fractions, respectively, and 549,000 U/mg wet wt. bacteria for controls not treated with 2-mercaptoethanol. (5pp)

L7 ANSWER 38 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1991-04986 BIOTECHDS
TITLE: Novel recombinant tissue plasminogen-activators produced in Escherichia coli;
tissue plasminogen-activator gene cloning and expression;
protein engineering (conference paper)
AUTHOR: Saito Y; Sasaki H; Hayashi M; Suzuki S; Ishii Y; Koyama S
CORPORATE SOURCE: Fujisawa-Pharm.
LOCATION: Product Development Laboratories, Fujisawa Pharmaceutical Company, Limited, 1-6, 2-Chome, Kashima, Yodogawa-ku, Osaka 532, Japan.
SOURCE: Ann.N.Y.Acad.Sci.; (1990) 613, 452-54
CODEN: ANYAA9
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 1991-04986 BIOTECHDS

AB New recombinant tissue ***plasminogen*** - ***activator*** (t-PA) analogs were produced in Escherichia coli by protein engineering ***methods*** . Vectors were constructed for t-PA and its analogs (n-tPA, K1K2P with kringle-1, kringle-2 and protease domains, K2P with kringle-2 and protease domains, and D-n-tPA, D-K1K2P and D-K2P, which were point mutants of t-PA, K1K2P and K2P, respectively). Production of the proteins was dramatically improved by: removal of the 3'-noncoding region of t-PA cDNA from vectors; and expression in a heat shock induction resistant mutant of ***E*** . ***coli*** HIB101. The recombinant proteins were precipitated as monomers in cells, and were ***renatured*** by extraction with 8 M ***urea*** followed by dialysis against glutathione. Mutation of Arg-275 (cleavage site with plasmin, EC-3.4.21.7) caused an increase of catalytic enhancement by fibrin and decreased interaction with ***plasminogen*** - ***activator*** -inhibitor. K2P and D-K2P had longer half-lives in blood, thrombolytic activity on whole blood and Chandler thrombus, and efficacy with bolus or infusional administration in a rabbit thrombus model. D-K2P was more effective than t-PA in a dog with coronary thrombosis. (1 ref)

L7 ANSWER 39 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1990-06234 BIOTECHDS
TITLE: Production and characterization of a novel tissue-type plasminogen-activator analog in recombinant Escherichia coli; protein engineering (conference abstract)
AUTHOR: Ishii Y; Saito Y; Sasaki H; Hayashi H; Suzuki S; Koyama S
CORPORATE SOURCE: Fujisawa-Pharm.
LOCATION: Product Development Laboratories, Fujisawa Pharmaceutical Co. Ltd., Japan.
SOURCE: Protein Eng.; (1990) 3, 4, 381
CODEN: PRENE9
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 1990-06234 BIOTECHDS
AB Recombinant Escherichia coli is a more economical source than mammalian cell cultures for production of proteins such as tissue-type ***plasminogen*** - ***activator*** (tPA). However, it is difficult to produce active native tPA (n-tPA) using recombinant ***E*** . ***coli*** in high yield. tPA analogs which can produce active tPA were investigated using recombinant ***E*** . ***coli*** . The plasmin cleavage site, Arg275, of analog DK2P, comprising Kringle 2 and the protease domain of n-tPA, was replaced with Asp. The recombinant proteins were precipitated as an inactive form in the cells. The yield of active DK2P was enhanced to about 150 mg/l by optimization of ***renaturing*** conditions. It seemed that the difference in the number of disulfide bonds between DK2P and n-tPA affected the ***renaturation*** yield. The ***purification*** of active DK2P involved a very simple 3-step procedure comprising ionexchange chromatography twice and p-aminobenzamidine-Sepharose chromatography once. The total yield of the ***purification*** ***process*** was about 55%. This novel tPA analog was superior to n-tPA in terms of production costs and several biological properties. (0 ref)

L7 ANSWER 40 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1990-02339 BIOTECHDS
TITLE: Purification of urokinase-like enzyme precursor; pro-urokinase purification from recombinant Escherichia coli by solvent extraction and fractional elution
PATENT ASSIGNEE: Toyo-Soda
PATENT INFO: JP 01256386 12 Oct 1989
APPLICATION INFO: JP 1988-84851 6 Apr 1988
PRIORITY INFO: JP 1988-84851 6 Apr 1988
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 1989-344189 [47]
AN 1990-02339 BIOTECHDS
AB A ***urokinase*** (EC-3.4.21.31)-like enzyme precursor (***pro*** -UK) is ***purified*** by adsorption with alkyl group-bonded polymer gel, and then by fractional elution. More specifically, the ***pro*** -UK gene is integrated into the genome of recombinant Escherichia coli

cells. Fractional elution of ***pro*** -UK proceeds by regulating the concentration of an organic solvent, belonging to the amine series, in the eluate. Using this ***process*** , recombinant ***pro*** -UK can be ***purified*** and contaminating proteins derived from the host cells or pyrogens can be removed. In an example, ***E*** . ***coli*** cells were homogenized and ***pro*** -UK was extracted using a 5% monoethanolamine aq. solution containing cysteine. The pH was adjusted to 9.5, and 14 g Na2SO4 and 10 g celite were added. After stirring at RT for 30 min, the solution was filtered. The filtrate was applied (10 ml/min) to a column packed with straight chain, butyl group-bonded butyl Toyopearl, equilibrated with 8% Na2SO4 aq. solution. The column was then washed and ***pro*** -UK was eluted by 5% monoethanolamine-HCl buffer. The specific activity of the recovered ***pro*** -UK was 57,000 U/mg and 66% activity was recovered. (4pp)

L7 ANSWER 41 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1990-01441 BIOTECHDS
TITLE: Purification of recombinant urokinase precursor; by cation-exchange chromatography
PATENT ASSIGNEE: Toyo-Soda
PATENT INFO: JP 01247087 2 Oct 1989
APPLICATION INFO: JP 1988-75083 29 Mar 1988
PRIORITY INFO: JP 1988-75083 29 Mar 1988
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 1989-330032 [45]
AN 1990-01441 BIOTECHDS
AB A ***method*** for ***purification*** of recombinant ***urokinase*** (EC-3.4.21.31) is claimed, involving adsorption on a cation-exchange resin and elution. This ***method*** allows high ***purification*** of recombinant ***urokinase*** , and contaminating proteins and pyrogens from the host cells can be removed. In an example, recombinant ***E*** . ***coli*** containing a human gene encoding ***urokinase*** was cultured, and a cell extract was prepared in 4 M ***guanidine*** -HCl and ***diluted*** 4 times in the presence of glutathione for protein ***renaturation*** . The enzyme was then ***purified*** by ammonium sulfate precipitation, dialysis and ionexchange chromatography on SP-Toyopearl 650 S. The specific activity (86,000 U/A260) was increased 3-fold over the original solution, and recovery was 31%. (4pp)

L7 ANSWER 42 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1989-13459 BIOTECHDS
TITLE: Activation of insoluble recombinant protein; protein renaturation and protein folding using alcohol; somatotropin, insulin, pro-urokinase or tissue plasminogen-activator gene cloning and expression in Escherichia coli
PATENT ASSIGNEE: Toyo-Soda
PATENT INFO: JP 01168298 3 Jul 1989
APPLICATION INFO: JP 1987-323764 23 Dec 1987
PRIORITY INFO: JP 1987-323764 23 Dec 1987
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 1989-232141 [32]
AN 1989-13459 BIOTECHDS
AB A ***method*** is claimed for activation of ***insoluble*** recombinant protein, involving addition of a hydrophilic alcohol organic solvent to a modified and ***solubilized*** protein-containing solution. The ***process*** is effective for re-folding of ***insoluble*** , incorrectly folded protein, produced in a heterologous host. High yield activation of recombinant protein is possible by the ***process*** . The recombinant protein is preferably human somatotropin, human ***pro*** - ***urokinase*** (EC-3.4.21.31), human tissue ***plasminogen*** - ***activator*** , or cattle somatotropin, produced in transformed Escherichia coli host cells. The hydrophilic alcohol solvent may be a monohydric or polyhydric alcohol with 1-12 carbons, e.g. methanol, isopropanol, ethanol or butanol. For activation of ***pro*** - ***urokinase*** produced in ***E*** . ***coli*** , the ***pro*** - ***urokinase*** is modified and

solubilized with ***guanidine*** hydrochloride and ***diluted*** , and 2-10 wt.% of the solvent is added to the ***diluted*** protein solution. (3pp)

L7 ANSWER 43 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1989-08182 BIOTECHDS
TITLE: Synthesis and purification of active human tissue plasminogen-activator from Escherichia coli; vector plasmid pXL348, plasmid pXL459, plasmid pXL130 construction
AUTHOR: Sarmientos P; Duchesne M; Denefle P; Boiziau J; Fromage N; *Cartwright T
CORPORATE SOURCE: Rhone-Poulenc
LOCATION: Laboratoire de Biochimie des Macromolecules, Institut de Biotechnologie, Centre de Recherches de Vitry, Rhone-Poulenc Sante, BP 14, 94403 Vitry sur Seine Cedex, France.
SOURCE: Bio/Technology; (1989) 7, 5, 495-501
CODEN: BTCHDA
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 1989-08182 BIOTECHDS
AB Since there is a need for a more economical source of human tissue ***plasminogen*** - ***activator*** (tPA) than mammalian cell lines, a bacterial expression system was investigated. Human tPA cDNA was cloned from the Bowes melanoma cell line. High copy-number derivatives of plasmid pBR322, vector plasmid pXL348 and plasmid pXL459, were constructed by fusion of the Met-tPA or pre-tPA coding sequence to the translation initiation region derived from phage lambda cII gene, but with the tR1 transcription terminator deleted (phage lambda-tR1 cII RBS), and under the control of the Ptrp promoter. Expression levels corresponding to about 5-7% of total proteins were obtained in Escherichia coli K12 C600 or ***E*** . ***coli*** B. Vector plasmid pXL130 was constructed with the Met-tPA coding sequence under the control of the PL promoter. The best expression levels were obtained in N+ strains when the gene was fused to a non-deleted version of the cII RBS. In all cases, tPA was mostly expressed as an ***insoluble*** ***denatured*** protein. A ***process*** was developed that allowed the recovery of significant amounts of fully active tPA, and its ***purification*** and characterization. (41 ref)

L7 ANSWER 44 OF 47 LIFESCI COPYRIGHT 2005 CSA on STN
ACCESSION NUMBER: 88:78885 LIFESCI
TITLE: Enhanced expression of human pro-urokinase cDNA in Escherichia coli .
AUTHOR: Hibino, Y.; Miyake, T.; Kobayashi, Y.; Ohmori, M.; Miki, T.; Matsumoto, R.; Numaro, N.; Kondo, K.
CORPORATE SOURCE: Tokyo Res. Inst., Central Glass Co., Ltd., Imafuku Nakadai 2805, Kawagoe, Saitama 356, Japan
SOURCE: AGRIC. BIOL. CHEM., (1988) vol. 52, no. 2, pp. 329-336.
DOCUMENT TYPE: Journal
FILE SEGMENT: J
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Human ***pro*** - ***urokinase*** cDNA was isolated from the cDNA library constructed from human kidney mRNA using the dC/dG homopolymer tailing ***method*** and Okayama-Berg ***method*** with pBR322 as a vector. A mature polypeptide starting with Ser was produced in Escherichia coli under the control of the tac promoter and the Shine-Dalgarno sequence of the catechol 2,3-oxygenase gene derived from Pseudomonas putida . By replacing the sequence coding for N-terminal eight amino acids of ***pro*** - ***urokinase*** with the synthetic DNA oligomer, the bacterial ***pro*** - ***urokinase*** had a molecular weight of 47,000 daltons and accounted for 15% of the ***insoluble*** fraction of ***E*** . ***coli*** proteins in induced cells. Its biological activity was restored by ***renaturing*** the bacterial product. The activity of bacterial ***pro*** - ***urokinase*** was 450 IU/ml culture.

L7 ANSWER 45 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1987-12901 BIOTECHDS

TITLE: Mature human tissue plasminogen-activator production; plasmid vector construction, cloning in Escherichia coli
PATENT ASSIGNEE: Genetica
PATENT INFO: EP 236209 9 Sep 1987
APPLICATION INFO: EP 1987-400354 19 Feb 1987
PRIORITY INFO: FR 1986-2380 21 Feb 1986
DOCUMENT TYPE: Patent
LANGUAGE: French
OTHER SOURCE: WPI: 1987-251699 [36]
AN 1987-12901 BIOTECHDS
AB The production of recombinant human tissue ***plasminogen*** - ***activator*** (t-PA) comprises growing a bacterium containing a plasmid encoding: the PL phage lambda promoter or the Ptrp promoter of the Escherichia coli tryptophan operon; the ribosome binding site of the phage lambda cII gene (optionally lacking the tR1 transcription terminator sequence); the t-PA gene containing a 5'-ATG start codon; and optionally the early termination region of phage T7. The new plasmids are termed plasmid pXL 130 and pXL 382. The new ***method*** gives t-PA expression 150-fold higher than previously described ***methods*** . Plasmid pXL 130 contains sequence tR1 but lacks the phage T7 sequence while the plasmid pXL 382 has the phage T7 sequence and lacks tR1. The inactive, ***insoluble*** form of t-PA expressed is converted to the mature form by a ***denaturation*** - ***renaturation*** ***process*** which permits rearrangement of the polypeptide structure. The plasmids are preferably cloned in ***E*** . ***coli*** cells, especially a strain which contains the cro gene of phage lambda. (41pp)

L7 ANSWER 46 OF 47 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1985-07002 BIOTECHDS
TITLE: E.coli clone producing human preprourokinase; thrombolytic production in Escherichia coli
PATENT ASSIGNEE: UCB
PATENT INFO: BE 900826 15 Apr 1985
APPLICATION INFO: BE 1984-900826 16 Oct 1984
PRIORITY INFO: BE 1984-900826 16 Oct 1984
DOCUMENT TYPE: Patent
LANGUAGE: French
OTHER SOURCE: WPI: 1985-105207 [18]
AN 1985-07002 BIOTECHDS
AB Production of an Escherichia coli clone which produces human preprourokinase (I) comprises first extracting and ***purifying*** total mRNA from human ***urokinase*** (II)-producing cells, e.g. pharyngeal carcinoma Detroit 562, and using the product for in vitro synthesis of complementary double-stranded DNA. This is enriched in components at least large enough to code for (II). The product is given cohesive ends, and inserted into plasmid pBR322 at the ampicillin-resistance gene PstI site. The hybrid molecules are used to transform ***E*** . ***coli*** and the transformants containing the DNA insert are selected to provide a clone bank. Clones containing (II)-coding inserts are isolated using a (II)-specific probe, DNA is extracted and inserted into plasmid pCQV2 at the BamHI site, and recloned in ***E*** . ***coli*** . Clones having DNA inserted in the correct orientation and reading frame are selected, and their ability to produce (I) is confirmed by immunoassay. The ***method*** allows (I), a thrombolytic, to be produced in large quantities. (23pp)

L7 ANSWER 47 OF 47 BIOTECHNO COPYRIGHT 2005 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1983:13144360 BIOTECHNO
TITLE: Purification of urokinase from complex mixtures using immobilized monoclonal antibody against urokinase light chain
AUTHOR: Vetterlein D.; Calton G.J.
CORPORATE SOURCE: Purif. Eng. Inc., Columbia, MD 21046, United States.
SOURCE: Thrombosis and Haemostasis, (1983), 49/1 (24-27)
CODEN: THHADQ
DOCUMENT TYPE: Journal; Article
COUNTRY: Germany, Federal Republic of
LANGUAGE: English
AN 1983:13144360 BIOTECHNO
AB The preparation of a monoclonal antibody (MAB) against high molecular

weight (HMW) ***urokinase*** light chain (20,000 M(r)) is described. This MAB was immobilized and the resulting immunosorbent was used to isolate ***urokinase*** starting with an impure commercial preparation, fresh urine, spent tissue culture media, or ***E*** . ***coli*** broth without preliminary dialysis or concentration steps. Monospecific antibodies appear to provide a rapid single step ***method*** of ***purifying*** ***urokinase*** , in high yield, from a variety of biological fluids.

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